Breeding Walnuts (*Juglans Regia*)

Gale McGranahan and Chuck Leslie

1 Introduction

1.1 Origin and History

Ancestral forms of walnut once spanned Europe, Asia, and the Americas as far north as Alaska. Climate changes altered the geographic distribution and further evolutionary pressures resulted in the 21 species of *Juglans* in existence today. All species produce nuts, but the Persian or English walnut (*Juglans regia*) is the only species widely cultivated for nut production and will be the focus of this chapter. Other species are grown for timber (e.g., *J. nigra*, eastern black walnut) or are used as rootstocks for Persian walnut (e.g., *J. hindsii*, northern California black walnut).

Persian walnuts are native to the mountain ranges of Central Asia extending from Xinjiang province of western China, parts of Kazakhstan, Uzbekistan, and southern Kyrgyzstan, and from the mountains of Nepal, Tibet, northern India, and Pakistan west through Afghanistan, Turkmenia, and Iran to portions of Azerbaijan, Armenia, Georgia, and eastern Turkey. Small remnant populations of *J. regia* may have survived the last glacial period in southeastern Europe, but the bulk of the wild *J. regia* germplasm in the Balkan Peninsula and much of Turkey was most likely introduced from Iran and eastern Turkey by Greek commerce and settlement several thousand years ago (Zohary and Hopf, 1993). From Greece, the cultivation spread to Rome, where walnuts were known as *Jovis Glans*, or Jupiter's acorn, from which comes the genus name *Juglans*. From Italy, *J. regia* spread to what are now France, Spain, Portugal, and southern Germany (Leslie and McGranahan, 1998). The word walnut may be derived from "wealh nut," "wealh" meaning foreign in Anglo-Saxon or old German. Trees of this species were in England by 1562, and nuts were brought

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to America by the earliest settlers. The American colonists are said to have called the species "English" walnut to distinguish it from the native American eastern black walnut (*J. nigra*). *J. regia* germplasm in China is thought to have been introduced from central Asia about 2000 years ago and in some areas became naturalized, although there appear to be natural stands in the Xinjiang Uygur Autonomous Region of China.

1.2 Production

Persian walnuts are grown in North, Central, and South America, Europe, Asia, and the former Soviet Republics, and to a limited extent in Oceania and North Africa. Over 1.4 million metric tons were produced in 2003 (FAOSTAT data, 2004). China leads world production, followed by the USA, Iran, Turkey, Ukraine, Romania, France, and India (FAOSTAT data, 2004). The major exporters are the USA, which exports 115,000 MT, followed by France (23,000 MT), China (22,000 MT), and India (17,000 MT). Shelled walnuts make up 62% of the exports. Several of the major producers consume the bulk of their walnut production domestically, for example, China, Iran, and Turkey. Chile, on the other hand, exported 13,000 MT in 2003, almost its entire production. China has encouraged its growers to plant high-value crops like walnuts and expects to have over 1 million hectares of walnuts by 2012. New areas of production are also developing in Chile and Argentina.

1.3 Uses and Nutritional Composition

Walnuts have had many uses over time. Although now the dried walnuts are consumed either as a snack or dessert nut or in baked goods, in times past they had a variety of uses. They were thrown by the grooms in Roman weddings to signify maturity. In the middle ages, they were used to ward off lightening, fevers, witchcraft, and epileptic fits. According to the Doctrine of Signatures (16–17th centuries), tinctures of the husk were used for ailments of the scalp and the kernel could be used to sooth the brain (Rosengarten, 1984). Currently, recipes can be found for green walnut pickles and walnut liqueurs, and in parts of the world, the undried walnuts, 'fresh walnuts', are eaten after peeling off the bitter seed coat.

Oils are the most prominent nutrient in walnuts (Tables 1 and 2). Recently, the health benefits of the oils, especially the omega-3 fatty acid, in walnuts have been investigated and found to be highly beneficial. In one study that compared a low fat and modified low diet, it was shown that including 8–10 walnuts per day improved the high-density lipoprotein (HDL) to total cholesterol in men and women diagnosed with type 2 diabetes. The low-density lipoprotein (LDL) was also decreased by 10% (Tapsell et al., 2004). In another study (Ros et al. 2004), a Mediterranean diet was compared to a similar diet in which

Table 1 Nutrient composition of walnuts

Nutrients		Amount in 100 g of kernel
Proximate	Water	4.07 g
	Food energy	654 kcal
	Protein	15.23 g
	Total lipid	65.21 g
	Carbohydrate	13.71 g
	Dietary fiber	6.7 g
	Ash	1.78 g
Minerals	Calcium	98 mg
	Copper	1.59 mg
	Iron	2.91 mg
	Magnesium	158 mg
	Manganese	3.41 mg
	Phosphorus	346 mg
	Potassium	441 mg
	Selenium	4.9 μg
	Sodium	2 mg
	Zinc	3.09 mg
Vitamins	Ascorbic acid	1.3 mg
	Thiamin	0.34 mg
	Riboflavin	0.15 mg
	Niacin	1.13 mg
	Pantothenic acid	0.57 mg
	Vitamin B6	0.54 mg
	Folate	98 μg
	Vitamin A	20 IU
	Vitamin E	0.70 IU

USDA National Nutrient Database for Standard Reference (2004)

Table 2 Walnut oil composition

Lipids			Amount in 100 g of kernel	
Fatty acids, total			62.23 g	(100%)
Saturated, total			6.13 g	(10%)
Palmitic		16:0	4.40 g	(7%)
Stearic		18:0	1.66 g	(3%)
Ecosanoic		20:0	0.06 g	(<1%)
Monounsaturated, total			8.93 g	(14%)
Gadoleic		20:1	0.13 g	(<1%)
Oleic		18:1	8.80 g	(14%)
Polyunsaturated, total			47.17 g	(76%)
Linoleic	(Omega-6)	18:2	38.09 g	(61%)
Linolenic	(Omega-3)	18:3	9.08 g	(15%)

USDA National Nutrient Database for Standard Reference (2004) walnuts (8–13) replaced approximately 32% of the energy from monounsaturated fat. The walnut diet increased endothelium-dependent vasodilation by 64% and reduced vascular cell adhesion molecule-1 by 20%. The diet also decreased total cholesterol and LDL cholesterol. Just recently (Reiter et al., 2005), a significant level of melatonin was identified in walnuts. According to the author R.J. Reiter, “the ingredients in walnuts would be expected to reduce the incidence of cancer, delay or make less severe neurodegenerative diseases of aging . . . and reduce the severity of cardiovascular disease.”

2 Botany

2.1 Taxonomy

The family Juglandaceae consists of seven genera and about 60 species of deciduous, monoecious trees with alternate, pinnately compound leaves. It has been extensively studied by Manning (1978) and Manos and Stone (2001). In addition to the genus *Juglans* (walnuts), the family includes *Carya* (pecans and hickories), *Pterocarya* (wingnuts), *Platycarya*, *Engelhardia*, *Alfaroa*, and *Oreomunnea*.

Members of the genus *Juglans* are trees or large shrubs possessing twigs with chambered piths, large aromatic compound leaves, generally solitary staminate catkins on 1-year-old wood, and female flowers on current season’s wood. The husked fruit is a false drupe containing a large, woody-shelled nut. All *Juglans* produce edible nuts, although size and extractability differ considerably. Most species are highly regarded for their timber.

The genus *Juglans* consists of approximately 21 species native to parts of North America, the Andean region of South America, and the mountain ranges traversing Central Asia (Table 3). These species have been grouped taxonomically into four sections: *Juglans*, *Trachycaryon*, *Cardiocaryon*, and *Rhysocaryon*.

Section Juglans. The *Juglans* section consists solely of the commercially valuable Persian or English walnut, *J. regia*. This section is characterized by a four-celled nut, a husk that separates from the nut at maturity, and seedlings with two rows of buds immediately above the cotyledons and below the spirally arranged compound leaves. The typically large tree grows to a height of about 30 m and produces large, relatively smooth, and generally thin-shelled nuts (Fig. 1).

J. regia selections have been identified in which nuts vary from nearly round to the greatly elongated ‘Barthere’ and from pea sized to more than 5 cm diameter. Trees with a weeping growth habit have been identified in Belgium and California, and variations in leaf morphology and color have been identified. Cutleaf types include ‘Heterophylla’ and ‘Laciniata.’ ‘Monophylla’ has leaves with only an enlarged terminal leaflet occasionally with two greatly reduced side leaflets; ‘Adspersa’ produces mottled white leaves, and ‘Purpurea’

Table 3 Species and their range in the genus *Juglans* (after Manning 1978)

Section and species	Common name	Range
Juglans		
J. regia L.	English or Persian walnut	Southeastern Europe, Iran to Himalayas, and China
Trachycaryon		
J.cinerea L.	Butternut	Eastern United States
Cardiocayon		
J. ailantifolia Carr. (*J. sieboldiana*)	Japanese walnut	Japan
var. *cordiformis*	Heartnut	Japan
J. cathayensis Dode	Chinese walnut	Eastern China, Taiwan
J. mandshurica Maxim.	Manchurian walnut	Manchuria, northeastern China, Korea
Rhysocaryon		
J. australis Griseb.		Argentina
J. boliviana		Western South America
(C. DC) Dode		
J. californica S. Wats.	Southern California black walnut	Southern California
J. hindsii (Jeps.) Rehder	Northern California, black walnut	Northern California
J. hirsuta Mann.		Northeastern Mexico
J. jamaicensis C. DC.	West Indies black walnut	West Indies
J. major		
(Torr. Ex Sitsgr.) Heller	Arizona black walnut	Southwestern United States, northwestern Mexico
var. glabrata Mann.		South-central Mexico
J. microcarpa Berl. (*J. rupestris*)	Texas black walnut	Southwestern United States, northwestern Mexico
var stewartii (Johnston) Mann.		Northern Mexico
J. mollis Englem. Ex Hemsl.		Central Mexico
J. neotropica Diels		Northwestern South America
J. nigra L.	Eastern black walnut	Eastern United States
J. olanchana		
Standl. and L.O. Williams		Guatemala
var. standleyi Mann.		Southeastern Mexico
J. pyriformis Liebm.		Southeastern Mexico
J. soratensis Mann.		Bolivia
J. steyermarkii Mann.		Guatemala
J. venezuelensis Mann.		Venezuela

Fig. 1 Walnuts on tree

exhibits leaves of a dull red color (Rehder, 1940). Cultivars with bright red seed coats have also been bred (McGranahan and Leslie, 2004).

The considerable variation within *J. regia*, particularly in nut size and shape, led taxonomists to describe six additional species that others have not accepted but which illustrate some of the diversity (Dode 1909). *J. sigillata* Dode, a type from southern China and Tibet with a very thick rough-shelled nut, an adherent hull, and very dark colored kernels, is the most distinctive of the variations described and is currently accepted as a separate species by some botanists. This status has been supported by recent isozyme analysis. Known locally as the iron walnut, this type or species has been cultivated for a long time in Yunnan Province for its oil, and several cultivars have been developed.

Section Trachycaryon. The Trachycaryon section consists only of *J. cinerea* L., butternut, a North American species, characterized by a two-chambered nut exhibiting eight prominent ridges on the shell and an indehiscent husk. The seedlings exhibit few if any scale buds immediately above the cotyledons, resulting in a long-naked area on the lower seedling stem where other species typically produce scale leaves. The nuts are borne in clusters of several nuts each on a long stalk, and the husks are conspicuously four-ribbed. Section Trachycaryon appears to be very closely related to the Asian section Cardiocaryon.

J. cinerea is native from New Brunswick to Georgia and west to Minnesota and Arkansas and is the most cold hardy of the North American walnuts. Also known as the white walnut or oil nut, this species is often found on river bottoms in mixed hardwood forests and will tolerate a high water table. Seldom found in pure stands and reaching a height of up to 30 m, it is a shorter, more spreading tree than *J. nigra* with which its range substantially overlaps. *J. cinerea* also has a relatively short lifespan, seldom living longer than 80–90 years.

Butternut wood is not as strong or durable as that of black walnut but is used for furniture, box, and toy construction. The kernels are large and in selected cultivars can be cracked out in halves. About 25 butternut cultivars have been selected from the native seedling population for their cracking characteristics, shell thinness, and yield. A few of these are cultivated, but no significant commercial use has developed and now butternut canker is decimating native stands.

Section Cardiocaryon. The Cardiocaryon section contains species that produce two-chambered nuts with 4–8 prominent ridges and indehiscent husks, and the nuts are borne in racemes of 5–25 nuts each. Seedlings exhibit five rows of scale buds immediately above the cotyledons, which merge into small alternate compound leaves higher on the stem. Members of this section are native to eastern Asia where their nuts and timber are utilized, but their susceptibility to walnut bunch disease has limited their horticultural development in the eastern USA.

J. ailantifolia Carr., the Japanese walnut reaches a height of 25 m, has leaves that are very pubescent on the lower surface, and bears its nuts in long racemes of up to 20 nuts each. This species is native to Japan where trees are generally found along streams and in moist plains. Although nuts of *J. ailantifolia* are typically difficult to crack, a seedling variant known as the heartnut, *J. ailantifolia* var. *cordiformis* (Maxim.) Rehd., bears heart-shaped nuts that crack more easily and from which kernels can be removed whole.

J. mandshurica Maxim., the Manchurian walnut, can grow to 30 m in height and 50 cm in diameter. Nuts are borne in clusters of five to seven nuts each on short, 10–15 cm, pendulous racemes. This species, native to northeastern China, Manchuria, and Korea, is the most cold-hardy of the *Cardiocaryon* but the nuts are difficult to extract. *J. mandshurica* is used in China mainly for timber and furniture and as a rootstock in cold areas of northern China. The nuts are highly variable in size and shell thickness.

J. cathayensis Dode, the Chinese walnut or Chinese butternut, is a vigorous tree or shrub up to 25 m in height. The small, edible nuts with very hard shells are produced on pendulous or erect spikes 8–15 cm long, which bear 6–13 flowers each. This species is native to much of central and eastern China and is thought to be botanically very close to *J. mandshurica*, perhaps a geographical variant, *J. cathayensis* var. *formosana* Hayata, native to Formosa and the southern portions of the *J. cathayensis* range, exhibits a smoother shell. *J. cathayensis* is reportedly a commonly used rootstock in regions of China along the Yangtze River.

Section Rhysocaryon. The Rhysocaryon section consists of approximately 16 North and South American *Juglans* species all of which exhibit four-chambered nuts with indehiscent husks, sutures that are not widened or winged, and shells that are ridged or striate but not completely smooth. Five rows of scale leaves at the base of seedling stems merge into small, spirally arranged compound leaves farther up the stem. *Juglans* species belonging to the section Rhysocaryon are found in much of the eastern USA and in localized portions of the west and southwest. Latin American members are found mostly in the mountains of Mexico, Central America, and the Andean region of South America with little geographical overlap of species ranges (Manning 1978). The species of this section are so closely related that it is often difficult to distinguish them, and generally nuts are so similar that they are of little value in separating species. Incomplete collection, considerable loss of the material that has been collected, and difficulty to travel in many of the remote mountainous areas where these trees are native have seriously impeded the study of both the taxonomic and economic characteristics of these species.

J. nigra L., the eastern black walnut, is the largest of the North American walnuts, reaching a height of 45 m and a trunk diameter of 2 m. It is native to the deciduous forests of the eastern USA and Canada where it is found most frequently in mixed stands on bottomlands and lower slopes with moist, well-drained soils. Eastern black walnut bears nuts with hard, black shells and stronger flavored kernels than those of *J. regia*. The irregular grooves and ridges on the shell separate it from the other species native to the USA, which produce evenly grooved to nearly smooth nuts.

Among wild *J. nigra* seedlings, there is considerable genetic variation in nut quality, blooming date, leafing date, age of first bearing, and growth rate. Over 400 cultivars of *J. nigra* have been selected for yield, nut characteristics, and timber quality. The best cracking black walnuts are the single-lobed sports, sometimes called peanut type, which have only a half nut and can be extracted whole. Examples include ‘Throp’, ’Blaettner’, and ‘Worthington’. Selections exhibiting lateral bud fruitfulness and up to 36% kernel have also been reported. ‘Deming Purple’ has reddish shades in its foliage, and a purple pellicle and ‘Laciniata’ is a variant with deeply indented foliage.

The high-quality wood is prized for furniture and gunstocks and is the most valuable hardwood produced in the USA. Eastern black walnut is now planted in Europe as both a timber species and a rootstock. The nuts, used for candies, baked goods, and ice cream, are scarce and generally more expensive than those of the Persian walnut. Almost all eastern black walnuts are harvested from wild trees without the aid of mechanical harvesting, and kernel yield averages only 8–25%.

J. hindsii (Jeps.) Rehder, the northern California black walnut, was once considered a variety of. *J. californica* S. Wats., but is now considered a separate species. The tree grows to a height of 30 m and produces round, smooth, hard-shelled nuts which vary considerably in size and quality but are generally smaller and less strongly flavored than those of *J. nigra.* At the time of European settlement, *J. hindsii* existed in only a few small groves in northern California. Although the species is a common shade tree in the region now, many of the existing trees may be hybrids with other black species. The most important use of *J hindsii* is as a rootstock alone or in the hybrid rootstock Paradox (*J. regia*×*J. hindsii*).

Other species in the Rhysocaryon section may be important locally and some have been tested as rootstocks to a limited degree. Recent work on the Paradox rootstock suggests that the Paradox rootstock commercially available may have hybridized with black species other than *J. hindsii.*

Other genera. Among the other genera of Juglandaceae, only the wingnuts (*Pterocarya*) have shown any promise of contributing to *Juglans* production. *Pterocarya stenoptera* C. DC., a vigorous colonizer of river banks and moist alluvial soils in its native China, exhibits a number of desirable rootstock properties, including considerable tolerance to *Phytophthora*, waterlogging, and nematode damage, but is incompatible with some cultivars of *J. regia* (McGranahan and Catlin 1987).

2.2 Interspecific Hybrids

Many of the species of this genus are capable of hybridizing with each other. In general, the black walnuts of section Rhysocaryon will not cross with species of sections Trachycaryon or Cardiocaryon, but *J. regia* will cross, at least to some extent, with members of the other three sections. The ability of *J. nigra* to cross with *J. ailantifolia* is an apparent exception to this generalization. The hybrid of greatest commercial importance is *J. regia*×*J. hindsii,* known as 'Paradox' and covered under the rootstock section of this chapter. Royal hybrids (*J. hindsii*×*$J. nigra*) are less vigorous than Paradox, perhaps due to their crop load, and are not used as rootstocks. Other *Juglans* hybrids with named cultivars include *J. cinerea*×*J. ailantifolia* crosses known as 'butterjaps' or 'buartnuts' and a *J. nigra*×*J. ailantifolia* cross named 'Leslie Burt', which exhibits anthracnose resistance. Although the native ranges of *J. nigra* and *J. cinerea* overlap substantially, the absence of confirmed hybrids suggests that these species are intersterile. In China, hybrids of *J. regia*×*J. mandshurica*, formerly the species *J. hopeiensis* Hu, are native to northern Hebei province near Beijing in northeast China.

2.3 Reproductive Biology

All *Juglans* species examined have 32 (2*n*) chromosomes and are monoecious, i.e., the male and female flowers are borne separately on the tree. The male flowers are densely packed on catkins that hang from the tree in the spring. Each catkin has up to 40 sessile petaless florets each with numerous stamens. The immature naked catkin buds first appear in leaf axils in late summer and persist over winter maturing in the spring in the axils of leaf scars on wood from the previous season. Female flowers are borne on current season's growth in spikes of two (to five) flowers in *J. regia* and more in other species. Flowers are typically produced on the tips of terminal shoots shortly after leaves emerge (Fig. 2). In some cultivars, female flowers are also produced on the tips of lateral shoots. This type of flowering is termed 'lateral bud fruitfulness' and is associated with high yields when trees are young. Lateral buds are rare on mature trees. The female flower consists of a hairy involucre fused to four sepals enclosing the pistil, which has a swollen base, the ovary, and a short style with a forked stigma with two feathery stigmatic lobes. The ovary is surrounded by the ovary wall, and inside the single locule is divided into four parts by the

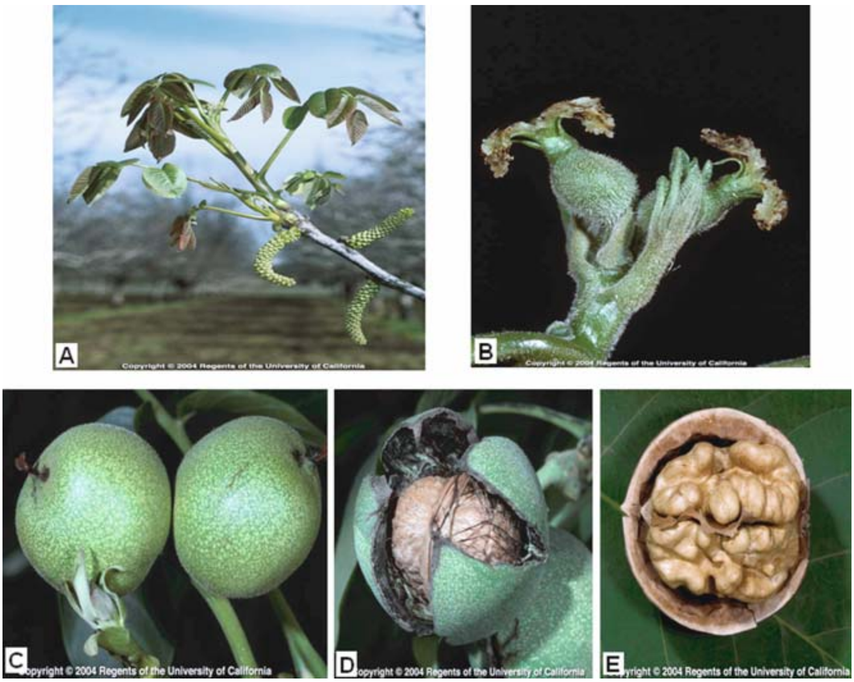

Fig. 2 (**A**) Walnut female flowers and catkins (male flowers); (**B**) flowers after pollination; (**C**) mature nuts in hull; (**D**) harvestable nuts; and (**E**) nut in shell

major and minor septa. Attached to the ovary is a single ovule enclosed by a single integuement (Polito 1998; Pinney et al. 1998).

Walnuts are heterodichogamous with male and female flowering occurring at different times. Some cultivars are protandrous, with the male flowering first, while others are protogynous, with the female flowering first. There is usually some overlap between male and female bloom, and walnuts are self-fertile, but the dichogamy promotes outcrossing.

Flowering occurs in the spring from mid-April for early cultivars to mid-May for late-blooming cultivars. Pollen is wind-borne and can be carried great distances, but is relatively short-lived. The female is receptive when the two stigmatic surfaces separate to form a V-shape and secrete an exudate that makes them appear moist. Once the lobes have achieved a 45-degree angle, the surface begins to dry, and the female flower is no longer receptive. If it has not been fertilized, the flower will expand for the next 3 weeks to the size of a marble and abscise. The stigma surfaces stay moist if the flower has not been fertilized. Another cause of pistillate flower abscission (PFA) is caused by excess pollen (McGranahan et al. 1994a). In this case, the flowers shrivel and abscise shortly after they lose receptivity. PFA occurs to some extent in all cultivars but is most serious in the cultivar ‘Serr’. The nut is harvestable approximately 19–22 weeks after bloom.

Timing of leaf emergence and harvest has always been an important consideration in selecting walnut cultivars. Early-leafing cultivars are more susceptible to frost and, in California, to walnut blight and insect problems. But early-leafing and flowering cultivars usually harvest early (in September) and have the advantage of early entry into the holiday nut trade. Late-harvesting (mid-October) cultivars can be subject to difficulties such as rain, which can interfere with harvesting operations. Vigorous late-harvesting cultivars that continue growing late into the autumn may also be more prone to injury by early frosts and winter injury.

3 Breeding

3.1 Objectives

The major breeding objectives are to increase yield, quality, and range of harvest dates while decreasing the amount of chemical input required to control pests and diseases (Table 4). The ideal walnut cultivar would be relatively late leafing to escape frost and the rains that spread walnut blight (*Xanthomonas campestris* pv. *juglandis*), precocious (yielding more than 500 kg/ha in the fourth year), and vegetatively vigorous with bearing on both terminal and lateral shoots. It would have a low incidence of PFA and other drops and would not be alternate bearing. It would have high production capacity (>6 MT/hectare) with low chemical input required. The harvest season would end in early October. The nutshell would be relatively smooth, well sealed, and make up no more than 50%

Table 4 Pests and diseases of walnut. (For more information see Ramos (1998) or IPM (2003))

Common name	Causal agent	Parts affected	Symptoms and signs	Importance	Control
Bacterial diseases					
Walnut blight	*X. campestris* pv. juglandis (Pierce) Dye	Leaves, shoots, husk, nuts	Spots (coalescing)	Major, widespread	Copper spray
Crown gall	Agrobacterium tumefaciens (Smith & Townsend) Conn	Roots, crown	Galls	Major	Sanitation, gall removal
Deep bark canker	*Brennaria rubrifaciens* Wilson et al.	Trunk	Cankers	Minor	Rootstock selection
Shallow bark canker	*Erwinia nigrifluens* Wilson, Star & Berger	Trunk	Cankers	Minor	
Fungal diseases					
Anthracnose	*Gnomonia leptostyla* (Fr.) Ces. et de Not (*Marssonina juglandis*) (Lib.) Magn. (anamorph)	Leaves, shoots, husk, nut	Spots (coalescing)	Major but not in California	Copper spray
Armillaria root rot	*Armellaria mellea* (Vahl:Fr.) *P. Kumm.*	Roots, crown	Rot, mycelial fans rhizomorphs	Minor, severe in some locations	Sanitation
Phytophthora root and crown rot	*Phytophthora* spp.	Roots, crown	Rot, cankers	Major	Rootstock selection
Butternut canker	*Sirococcus clavigignenti-juglandacearum*	Trunk, branches	Cankers	Only in butternut	
Viral diseases					
Blackline disease	Cherry leafroll virus (CLRV)	Systemic in *J. regia*	Blackline at graft union between *J.*	Major	Tolerant rootstocks

Table 4 (continued)

Common name	Causal agent	Parts affected	Symptoms and signs	Importance	Control
			regia and hypersensitive rootstocks		
Nematodes					
Root lesion nematode	*Pratylenchus vulnus* Allen & Jenson	Roots	Black lesions on roots, dead feeder roots	Major	
Root knot nematode	*Meloidogyne* spp.	Roots	Swellings on roots	Limited to coarse soils	Sanitation, soil fumigants
Ring nematode	*Mesocriconema xenoplax* (Raski) Luc and Raski	Roots	Stunted feeder roots	Minor	Sanitation, soil fumigants
Insects					
Codling moth	*Cydia pomonella* L.	Husk, nut	Premature abscission, frass at entry hole	Major, increases susceptibility to navel orange worm	Insecticide sprays, pheromone mating disruption predators and parasites
Navel orange worm	*Amyelois transitella* Walker	Husk, nut	Wormy nuts, frass inside nut	Major	Prompt harvesting, insecticide sprays
Walnut husk fly	*Rhagoletis completa*, Cresson	Husk	Wormy husk, shell stains	Major	Insecticide sprays
Mites, aphids, and scales					
Walnut aphid	*Chromaphis juglandicola*, Kaltenbach	Leaves	Feeding aphids, leaf abscission	Minor with flare ups	Parasitic wasp: *Trioxys pallidus*

of the nut weight. The nuts would fit the category of large or jumbo. The kernel would be plump and light colored, weighing about 8–9 g, and come out easily in halves. The tree would be at least moderately resistant to pests and diseases.

3.2 Genetic Resources

A breeding program depends in part on a diverse collection of germplasm as a source of raw material from which traits of interest can be identified (McGranahan and Leslie 1990). For the past two decades, extensive evaluations of seedlings in orchards and naturalized trees have been undertaken in the Mediterranean countries of Europe and to a lesser extent North Africa (see Proceedings of Walnut Symposia: Acta Horticulturae numbers (1990) **284**, (1993) **311**, (1997) **442**, (2001) **544,** and 2006 (705)). From this work, several new cultivars have been identified (Tomas 2000). China has also had an active nationwide search for new cultivars from seedling orchards. Because Persian walnuts are native to the mountains of Central Asia, considerable effort in the USA has been directed toward collecting material from that area (Leslie and McGranahan 1998). Funding and participation in this work have included a century-long plant introduction endeavor by United States Department of Agriculture (USDA) plant collectors, and more recent trips by USDA and university researchers. Collecting has been funded in part by California growers, USAID exchanges, and USDA-ARS Germplasm exploration funds. Material has also become available for use through international germplasm exchanges, private breeders, hobbyists, customs confiscations, and observant growers in the state who have noticed interesting seedling trees.

A very useful book, "Inventory of Walnut Research, Germplasm and References," has recently been published by FAO and it describes a great number of germplasm collections in the world, especially in the European Union (Germain 2004). In the USA, both the University of California (UC) and the USDA National Clonal Germplasm Repository, Davis, California (NCGR-Davis), maintain walnut germplasm collections. The content at the Davis Repository walnut collection is listed at www.ars.usda.gov/main/www.ars-grin.gov/dav_main.htm?modecode = 53-06-20-00.

The intent of the USDA collection is to include as broad a diversity of all walnut species as possible and maintain it for public distribution of material. It will not accept proprietary material and is managed primarily for wood and nut distribution to researchers worldwide. The UC Davis collection includes a representation of California commercial varieties, advanced selections, and some proprietary material, and is focused primarily on material of interest for breeding purposes (Tulecke and McGranahan 1994). It is managed for a variety of activities, including crossing, breeding evaluations, and graftwood distribution of advanced selections. While there is some overlap of material, duplication is generally avoided, and the two collections are used cooperatively.

3.3 Evaluation

Germplasm in these collections has to be evaluated and characterized to determine its useful attributes. Descriptors for evaluating germplasm have been published (McGranahan et al. 1994b) by the International Bureau of Plant Genetic Resources (formerly International Plant Genetics Resources Institute). The *Inventory of Walnut Research, Germplasm and References* (Germain 2004) includes evaluations of the primary international cultivars. The USDA Germplasm Resources Information Network (GRIN) has descriptions of some of the germplasm held at the NCGR (www.ars-grin.gov/). Evaluation of the UC Davis collection is primarily for the UC Davis breeding program, and the traits evaluated are shown (Table 5). In addition, the UC Davis collection has been evaluated for allergenic proteins to determine whether a nonallergenic walnut could be bred, but all the germplasm contained allergenic proteins (Comstock et al. 2004). Susceptibility to *Aspergillus flavus* and aflatoxin contamination was also evaluated. Susceptibility was comparatively low in walnuts compared to other nut crops, but there was significant variation when artificially inoculated; for example, 'Chico' had a much higher level of aflatoxin than 'Tulare' (Mahoney et al. 2003). Both stem-end hole size and degree of hull pubescence were evaluated to determine their effects on codling moth. Both traits showed significant variation, but only pubescence affected the codling moth by slowing its movement across the hull, allowing predators more time to kill the larvae. However, it was determined that no germplasm was sufficiently pubescent to have a major impact on codling moth infestation (unpublished data).

Table 5 Cultivar traits under evaluation and estimated heritabilities (Hanche et al. 1972)

Field	h^2	Crack out	h^2
Leafing date	0.96	Shell texture	
Female bloom: first, peak, and last	0.93	Shell color	
Male bloom: first, peak, and last	0.8	Shell seal	0.38
Dichogamy		Shell strength	
Percent overlap: male and female		Shell integrity	
Catkin abundance		Shell thickness	0.91
Female flower abundance		Packing tissue thickness	
Percent fruitful laterals	0.39	Nut weight	0.86
Yield	0.07	Kernel weight	0.87
Blight		Percent kernel	
Codling moth		Fill	
Sunburn		Plumpness	
Harvest date	0.85	Ease of kernel removal	
		Color (extra light, light, light amber, amber)	0.52
		Shrivel	
		Veins	0.49

Heritabilities are high for many traits of interest (Hanche et al. 1972; Forde and McGranahan 1996) (Table 5). However, it has been shown that many traits change with clone age; for example, leafing out, bloom, and harvest date all shift to 2 weeks earlier, stabilizing at age 15. Shells also thicken and seals improve, but the in-shell weight, kernel weight, and percent kernel all decrease (McGranahan and Forde 1985).

3.4 Crossing Methods

The UC breeding program has used two distinct procedures for crossing parent material. In the first method, wind-blown pollen is excluded from female flowers of interest by covering them with tightly secured bags that have small plastic windows. Pollen is collected from the other parent of interest and stored frozen over saturated magnesium chloride until use. When bagged female flowers open and are receptive, pollen is applied through the bags with a hypodermic needle. Bags are later removed and nuts marked for collection in the fall. The male parent is known with this method, but the costs are high and seedling production is low.

The second method is to locate geographically isolated young trees of the desired female parent. Using young trees is important, because as a cultivar matures, the female flowers are usually present 2–3 years before the male flowers. This often requires the cooperation of a grower with a recently planted orchard. Any male flowers on these trees are removed by hand before bloom to prevent selfing. Once the female flowers begin to bloom, pollen of the desired male parent or parents is applied by airbrush several times during the bloom period. At harvest, the cooperating grower either donates or is compensated for the nuts. This method produces many more seed at lower cost but with low certainty of the male parent. Male parents of selections can be determined later by DNA analysis. Some selfing also occurs, which results in stunted, twisty trees with russetted hulls and small kernels.

3.5 Seedling Evaluation

Seed collected from these crosses is then stratified and grown to produce the next generation of seedlings. These are screened as they mature for traits of interest (Forde and McGranahan 1996). Commercial walnut nurseries have generously donated growing ground, time, resources, and expertise to assist this aspect of the program.

After 1 year in the nursery, trees are dug and replanted on wider spacing for evaluation. At this stage, trees are grown on their own roots, not grafted to rootstock. Most commonly, these trees are planted on UC Plant Sciences Department growing grounds and farmed by department staff supported by university and grower funding. In some cases, growers have assisted the program by donating orchard space for this purpose and have farmed these trees during the evaluation process. This has been done by planting between rows in an existing widely spaced orchard, or more effectively, by interplanting in available open space in a newly established orchard and then removing the breeding program trees as evaluations are completed and the grower's orchard matures to fill the canopy.

As seedling trees mature, they are evaluated in the field for traits of interest, including leafing, flowering, and harvest dates, yield, and growth habit (Table 5). When the trees are grown in university orchards, they are left unsprayed so that variation in resistance to insects and disease can be observed. When grown within commercial orchards, this is not normally possible. Nut samples are hand collected from each tree at maturity. Samples are dried, cracked by hand, and evaluated for percentage of kernel, kernel quality, kernel weight, shell characteristics, and yield of halves (Table 5). Data are entered into a database and summarized for multiple years. In addition, samples of promising individuals are sent to commercial processors for their independent evaluation.

Collected data are presented to farm advisors, growers, and nurserymen in several ways. The first is at the annual Walnut Research Conference as part of the Walnut Improvement Program's annual report. Data on selections are presented orally to attendees and published in the annual proceedings of the conference (available from author).

The breeding program also holds an annual "Crackout Meeting" in the spring attended by farm advisors, handlers, nurserymen, and growers. Growers, handlers, and nursery attendees generally have an expressed interest in development of new varieties, are interested in assisting with evaluation of material, or are otherwise active in research activities and the marketing board. At this all-day meeting, the data reports are distributed, and kernel samples and intact nuts of the material under evaluation are displayed. Attendees are asked to review the material, examine the samples, and provide written comments. In an ensuing discussion period, they provide valuable input on priorities from their varying perspectives, help rank material, and suggest which should continue in the program. The program also regularly invites interested parties to view selections in the field, either through a formal field day or by scheduling informal visits at their convenience. Progress in the program and information about selections are also presented periodically to a wider range of growers at annual county grower meetings held around the state.

3.6 Selection Trials

Once an individual seedling shows promise and is selected for further trials, graftwood is collected from the original seedling and grafted to rootstocks.

Nurseries have often provided assistance at this stage by donating rootstock, supplying grafters, and, in many cases, growing the grafted trees for the program.

Grafted trees of each selection are then planted in test blocks on orchard spacing at diverse locations for further evaluation. Currently, these tests blocks are located at the Chico State University Farm in the northern part of the state, on the UC Davis campus in the central region, and at the UC Kearney Field Station in the south. These blocks are managed by cooperative extension farm advisors and are used to evaluate the performance of selections on rootstocks under a wide range of conditions, obtain a better look at yield, and allow farm advisors and growers to see selections in their local area.

In addition to the university plots, interested growers around the state have volunteered to establish trials ranging in size from several trees to several acres. Farm advisors assist in identifying suitable growers, establishing plots, and observing performance. Graftwood is distributed to these growers under test agreement, and they are asked to participate in its evaluation and to attend the *crack out meeting*. This gives the program valuable input on performance under a variety of conditions and in commercial settings from observers with extensive experience. Growers feel they are assisting the process and get an early look at the material that is most interesting for their situation.

As new selections begin to show promise, commercial nurseries are encouraged to acquire graftwood from the program to test the varieties for themselves and to begin increase blocks of their own. This ensures that nurseries have adequate input into final selection, firsthand knowledge of the material, particularly of its grafting performance, and growth habit and training requirements and build an adequate supply of production wood by the time the new variety is released. As with grower trials, nurseries receive wood under test agreement. This allows them to propagate for testing purposes, including grower trials, but trees cannot be produced for sale until they are patented.

3.7 Release of Selections

Selections that continue to show promise in test blocks and grower trials become candidates for patent and release as new cultivars. The patent disclosure process requires an extensive description of the selection, a summary of available data, and identification of attributes distinct from existing varieties.

Once a selection is patented as a new cultivar, nurseries may obtain a commercial license from the University of California that allows sale of trees. A per-tree royalty is assessed at the time of sale from the nursery and returned to the university. After patenting costs are recovered, part of this fee is assigned for overhead, and part is returned to the breeding program as well as the breeders.

Patenting provides a return to the inventor and the university but also seeks to protect the growers from unlimited distribution. Patented material is not allowed to be sold or grown outside of California for 5 years after release. After that period, overseas licensing provides a return to the program that would not otherwise occur.

3.8 Backcross Breeding for Hypersensitivity

Marker-assisted backcross breeding is being used to develop a commercial quality, *J. regia*-like cultivar with resistance (hypersensitivity) to the CLRV, which causes blackline disease (Woeste et al. 1996). We showed that a single dominant gene from *J. hindsii* confers hypersensitivity and that progeny from backcrosses (*J. hindsii* × *J. regia*) × *J. regia* segregates 1:1 hypersensitive–tolerant (McGranahan et al. 1997). Currently we are evaluating the BC4 generation. An anomaly in all the backcrosses is that they are male sterile, i.e., catkins, if formed, abscise when immature. We have selected three backcross genotypes, with close to commercial quality, for field trials. The field trials are designed to determine whether CLRV-infested pollen infects a hypersensitive flower, whether any damage to the flowers occurs at fertilization, and whether nut set is affected.

3.9 Breeding Accomplishments

Prior to the Serr-Forde breeding program (1948–1978) in California, most cultivars grown in Northern California, where the industry now resides, were cultivars brought from France by Felix Gillet in the late 1800s or chance seedlings. Gene Serr and Harold Forde made remarkable progress in breeding new cultivars that revolutionized the industry. Their primary breeding objectives were to combine the late leafing and quality of the French types with the lateral fruitfulness and precocity of ‘Payne’. They made 196 crosses, evaluated about 6000 progeny, and released 13 cultivars, 10 in 1968 and 3 in 1978. The most important of these are ‘Vina’, ‘Serr’, ‘Howard’, and ‘Chandler’ (Ramos, 1998). In 1993, ‘Tulare’ was released from a cross made 27 years earlier by Serr and Forde (McGranahan et al. 1992).

Recently, four new cultivars have been released. ‘Robert Livermore’ is a red-skinned walnut (McGranahan and Leslie 2004). ‘Sexton’, ‘Gillet’, and ‘Forde” (patent pending) are all precocious in bearing, laterally fruitful, high yielding, midseason harvesting, with low blight scores and high quality kernels. The latter two are protogynous, which is unusual in the cultivars available

4 Rootstock Improvement

The rootstock is the other half of the tree and provides anchorage, absorption of water and nutrients, hormone synthesis, and storage. Rootstocks are more difficult to study because they are mostly underground, and rootstock improvement is developing slowly because clonal propagation has not yet been commercialized in California. Traits of common rootstocks are shown in Table 6. Clearly genetic improvement is needed. To date, the Paradox rootstock (*J. hindsii* × *J. regia*), which exhibits hybrid vigor, is superior to pure species in most traits, but many other species combinations have not been tested (McGranahan and Catlin 1987). Paradox is seed propagated from *J. hindsii* (northern California black walnut) trees that are naturally pollinated by *J. regia* pollen.

Blackline tolerance. In California, we have approached the blackline problem, caused by the CLRV, through both cultivar hypersensitivity and rootstock tolerance. The latter is aimed at developing a rootstock combining the *J. regia* response to blackline disease with the vigor and other attributes of Paradox. This can be achieved, in theory, by selecting vigorous, tolerant individuals among seedlings of a backcross generation *(J. hindsii* × *J. regia)* × *J. regia*. In 1988, 13,000 Paradox offspring from 17 source trees were planted in a randomized complete block design with six blocks in *Phytophthora*-infested soil. Between 1992 and 1994, they were screened for vigor and tolerance to the virus. Five seedlings were selected in 1994, but it has taken until last year to establish grower trials to compare their performance in the field to Paradox and *J. regia* rootstocks because of the challenges of clonal propagation.

Nematode, crown gall, and Phytophthora resistance. A study to evaluate the diversity of Paradox rootstocks was initiated in 1996. It was designed to examine variability among families of commercially available Paradox seedlings and controlled crosses between different black walnut species and *J. regia*. Eleven California walnut nurseries each donated about 500 seeds from each of three Paradox-producing black walnut source trees each year for 2 years. These were planted in replicate blocks in three nurseries, measured and divided into subsets. Four subsets were planted and grafted as orchard trees (Wilbur Reil, Bob Beede, Joe Grant, Richard Buchner); two subsets were screened for nematode (*P. vulnus*) resistance by Michael McKenry (unpublished), and two were screened for crown gall (*A. tumefaciens*) resistance (McKenna and Epstein 2003). Two subsets of ungerminated seed were provided to Greg Browne for *Phytophthora* screening (Browne et al. in press).

The work is ongoing in the four long-term field trials, but in the process of screening seedlings for various traits, it became apparent that certain individual seedlings were superior. Two genotypes from the crown gall screen were selected; one proved to be an escape rather than a resistant genotype, and the other remains to be retested. Greg Browne has identified several genotypes that continue to have low susceptibility to *Phytophthora citricola* in repeated screens

Table 6 Walnut rootstock response to pests and diseases

Rootstock	Crown gall	Phytophthora root and crown rot	Blackline disease	Armillaria root rot	Root lesion nematode	Root knot nematode	Salts
Persian walnut (*J. regia*)	Susceptible	Very susceptible	Symptom less	Susceptible	Very susceptible	Susceptible	Sensitive
Northern California black walnut (*J. hindsii*)	Susceptible	Very susceptible	Hypersensitive	Variable	Susceptible	Resistant	Less sensitive
Paradox walnut (*J. hindsii* × *J. regia*)	Very susceptible	Susceptible	Hypersensitive	Variable	Very susceptible	Unknown	Sensitive
Wingnut (*P. stenoptera*)	Resistant	Resistant	Hypersensitive	Susceptible	Tolerant	Unknown	Unknown

For more information on pests and diseases see Ramos (1998) or IPM (2003) of micropropagated plants (Browne et al. in press). Mike McKenry found no resistance to nematodes but identified one genotype that did not appear to be affected by infestation (tolerant response). Most of the selected genotypes in this study have been micropropagated for field trials. These have been repropagated and are undergoing further field trials. It is expected that four or five new clonal rootstocks will be released from this study.

Much more work is needed on rootstocks. Since the hybrids appear to have the most vigor, it is important to evaluate the performance of different species in hybrid combinations. One that is readily available in South America and hybridizes easily with *J. regia* is *J. australis* from Argentina. Other possibilities are *J. neotropica* (northwestern South America) and *J. olanchana* (Mexico and Guatemala).

Clonal rootstock propagation. From the studies described above, it is clear that clonal rootstock is highly desirable. So far, only one commercial lab routinely produces micropropagated walnuts (Vitrotech Biotecnologia Vegetal, S.L., Murcia, Spain). Our lab has been successful in micropropagation, but we do not attempt it on a commercial scale. Micropropagation is accomplished by disinfestation and multiplication of nodal cuttings on gelled Driver-Kuniyuki-Walnut (DKW) medium (McGranahan et al. 1987). After initiation in vitro, shoots must be transferred frequently (two to five times per week) until the medium is no longer discolored by exudates. Multiplication occurs through axillary shoot proliferation and excision to initiate new cultures. Throughout the multiplication phase, walnuts have to be transferred relatively frequently. Cultures are maintained at room temperature under cool white fluorescent lighting.

A two-phase rooting system is used for micropropagated shoots (Jay-Allemand et al. 1992). Roots are induced by placing shoots on an auxin-containing medium in the dark. Induced shoots are then transferred to a vermiculite-gelled medium substrate and maintained in the light for 3 weeks. Rooted shoots are then transplanted to well-drained potting soil and acclimatized in a fog chamber in the greenhouse for 2 weeks, followed by a week or two under shade cloth. Dormancy can be induced once the plants have achieved 10–20 cm height by placing them at 10°C under short day length and low light intensity for 3 weeks. (Dormant plants can be stored for up to 6 months at 5°C.) Dormant plants are then planted in the nursery where they uniformly resume growth. A full description of the method will be published shortly (Leslie et al. in press).

5 Biotechnology

Many of the new tools of biotechnology have been applied to walnuts, as recently reviewed in Dandekar et al. (2005), but like many fruit and nut crops, walnuts lag behind the agronomic crops in this field. Gene transfer techniques have been in use for walnuts since 1988 (McGranahan et al. 1988), and field trials of mature transgenic trees are under way. Genes of interest include Bt from *Bacillus thuringiensis* for insect resistance (Dandekar et al. 1998; Leslie et al. 2001) and crown gall silencing for resistance to crown gall (Escobar et al. 2002). Tree architecture has been modified by the *rolABC* genes from *A. rhizogenes*, but the goal of increasing rootability was not achieved (Vahdati et al. 2002). When used as rootstock, the smaller stature and compressed internodes of the rol trees did not effect the phenotype of the scion. The reticence of the public to accept genetically engineered organisms has prevented any commercialization of transgenic walnut trees, but it is expected that transgenic rootstocks will prove more acceptable.

Marker-assisted selection is being used successfully in a backcross breeding program designed to transfer hypersensitivity to CLRV from *J. hindsii* into a commercially acceptable Persian walnut cultivar. This tool greatly reduces the time required to screen progeny in each generation. DNA finger printing is becoming routine for cultivar identification (Dangl et al. 2005), and DNA sequence markers were used to identify the species involved in a large study of Paradox seedlings (Potter et al. 2002). Gene cloning from walnuts is under way, and genes of interest include those that code for tannin, naphthaquinone, unsaturated fatty acid, and flavonoid biosynthesis. In spite of activity in walnut biotechnology, genome mapping will only take place in the distant future, when the tools are readily available and less expensive than they are today.

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