Juglans regia L: genetic variation and provenance performance.

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Thesis submitted for the degree of Doctor of Philosophy at the University of Oxford, Oxford, UK.

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Northmoor Trust

A superior walnut (*Juglans regia*) phenotype: tree 16 at Kyr-Sai (Provenance K10), Sary-Chalek Biosphere Reserve, Kyrgyzstan: tree height 26 m, height to first branch 17.7 m, *dbh* 36.7 cm, straightness rank 26, seeds collected 10.

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ABSTRACT

A range-wide collection of *Juglans regia* seeds was undertaken in autumn 1997 from 12 countries, including 25 provenances and 375 half-sib progenies. 2200 seedlings were produced using innovative nursery techniques. The seedlings were planted in three provenance trials in southern England in 1999, the largest of which acted as a combined provenance/progeny trial. After one growing season, survival was 98.9 %, mean height growth 35 cm, and mean stem diameter increment 5 mm. Provenance differences for both height and stem diameter increment were highly significant ($p<0.001$). There were no significant genotype × environment interactions. Flushing assessments revealed few significant differences between provenances and flushing was complete by early April. Family heritability for tree height was 0.19 at one site and, with combined selection, genetic gain was estimated at 8 %.

The effects of three types of treeshelter and a stumping treatment on walnut establishment were tested over three growing seasons. Treeshelters were found beneficial to height increment. However, 120 cm tall shelters promoted early flushing, and consequent risk of increased frost damage, and caused more stem die-back than 75 cm shelters. Stumping promoted rapid early height increment but gave no longer-term benefit. The crown (*cd*) and stem (*dbh*) diameter at breast height relationship of open growing trees in Britain was assessed and was highly significant ($r^2 = 0.96$, $p<0.001$). The regression equation ($cd = 2.71 + 17.6dbh$) permitted the estimation of suitable planting densities for the provenance trials and the calculation of a thinning regime.

Isozyme analysis of the 375 genotypes identified 20 loci in 15 enzyme systems with seed embryo extracts. Using young leaf extracts, the polymorphic locus *Pgm*-1 indicated low expected heterozygosity of 0.06 both within populations and at the species level. F_{ST} and G_{ST} estimates, both 0.05, indicated high uniformity among populations. Genetic distance estimates did not identify significant clustering consistent with geographic origin.

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Glossary

The following definitions, included to aid the reader in understanding the multidisciplinary text, were mostly copied or modified from the following references: Wright (1976), Williams and Matheson (1994), Lawrence (1995) and Helms (1998).

Allele - Alternative form of a gene at a locus.
Allele frequency - see Gene frequency.
Allozyme - Any of more than one variant of an enzyme encoded by different alleles at the same gene locus.
Anode - The positive electrode in an electrophoresis apparatus.
Analysis of variance (ANOVA) – A statistical analysis by which variance ratios are compared in such a manner as to determine the probability that differences among populations or treatments are too large to be due to chance.
ANOVA – see Analysis of variance.
Apomixis – A reproductive process without fertilisation in plants, including development from cells other than ovules.
Basal area – The cross-sectional area of all stems of a species or all stems in a stand measured at breast height and expressed per unit of land area.
Best Linear Unbiased Predictor (BLUP) - Values for terms specified as random effects in a mixed-effects model.
BLUP - see Best linear unbiased predictor.
Breast height – A standard height from ground level at 1.3 m for recording stem diameter, circumference or basal area of a tree.
Bud burst - see Flushing.
Bulked seedlots – Seedlots made up by mixing together seeds from different mother trees.
Burr – An irregular, commonly round growth on a tree stem or branch resulting from the entwined growth of a cluster of adventitious buds and having contorted grain.
Cathode - The negative electrode in an electrophoresis apparatus.
Cluster analysis - A method of hierarchically grouping taxa or sequences on the basis of similarity or distance.
Codominant alleles - Alleles of a given gene whose properties can be detected in a heterozygote.
Coppice – A plant derived by coppicing (the cutting of the main stem at the base to stimulate the production of new shoots).
Crown – The part of a tree or woody plant bearing live branches and foliage.
Cultivar – A clone, race or product of breeding selected from a population of plants because it has desirable characteristics and is generally more or less uniform.
Die-back – The progressive dying from the extremity of any part of a plant, which may or may not result in the death of the entire plant.
Dimeric - Active protein composed of two polypeptide chains.
Diploid - Having two sets of genes and two sets of chromosomes.
Drupe – A more or less fleshy fruit with one compartment and one or more stony seeds, having the pericarp differentiated into a thin epicarp, a fleshy mesocarp, and a hard stony endocarp.
Electrophoresis - A method of separating DNA or proteins according to their charge and/or size by placing a sample in an electrical field. DNA fragments are usually separated on either agarose or polyacrylamide gels, proteins on either starch or polyacrylamide.
Enzyme - A specialised protein which catalyses biochemical reactions.
Epistasis – An interlocus genetic interaction in which the expression of combinations of specific genes from different loci is not accurately predicted by a simple linear combination of their average effects.
F-statistics - A set of coefficients that describes how genetic variation is partitioned within and among populations and individuals.
Family – A group of individuals directly related by descent from at least one common ancestor.
Fixation index – see Inbreeding Coefficient.
Fixed effects – Model terms where a parameter is specified for each factor level, where inferences are made only about the experimental material itself and not about any hypothetical population of which the experimental material is a sample.
Flushing – The opening of buds and appearance of leaves.

Free-growth – A forest management system which stimulates vigorous crown development of selected trees by thinning, in order to achieve maximum radial stem increment.

GEI – see Genotype Environment Interaction.

Gene flow - The incorporation of genes into the gene pool of one population from one or more other populations.

Gene frequency - The proportion of gene copies in a population that an allele accounts for; *i.e*. the probability of finding this allele when a gene is taken randomly from the population. Equivalent to allele frequency.

Gene pool - The complete set of the genes of a given sexual population.

Gene - The functional unit of heredity.

Genetic distance - Any of several measures of the degree of genetic difference between populations, based on differences in allele frequencies.

Genetic drift - Random changes in the frequencies of two or more alleles or genotypes within a population.

Genome - This term may mean: (i) the genetic material of an organism; (ii) all the genes in a cell; (iii) the haploid set of genes or chromosomes in a cell.

Genotype - The genetic composition of an organism. That is, the organism's content of genetic information, either in total or with respect to or more particular named alleles, regardless of whether or not that information is, or can be, expressed under a given set of conditions.

Genotype Environment Interaction (GEI) – The differential response by genotypes to the environments in which they are grown.

Gibberellins (GA) – A group of substances affecting plant growth, believed to act as natural growth hormones.

Half-sib – Trees with one parent (usually the female) in common.

Haploid - A cell (or organism) having a ploidy of one.

Hardy-Weinberg (H-W) equilibrium - An equilibrium of genotypes achieved in populations of infinite size (in which there is no immigration, emigration, selection or mutation) after one generation of panmictic mating. With two alleles A and B of frequency p and q, the Hardy-Weinberg equilibrium frequencies for genotypes AA, AB and BB are p^2, $2pq$ and q^2 respectively.

Heritability – The proportion of variability of a character due to heredity, the remainder being due to environment. In a broad sense, that portion of the total variance due to all genetic factors. In a narrow sense, that portion of the total variance due to genes with additive effects and most indicative of the superiority that can be transmitted by sexual propagation.

Heritability, family – That portion of the total variance due to differences among families, and applicable only to family means.

Heritability, single tree – That portion of the total variance due to genetic differences among individuals, and applicable to data from single trees.

Heterozygote - The condition of having different alleles at corresponding loci on homologous chromosomes.

Homozygote - The condition of having the same alleles at corresponding loci on homologous chromosomes.

Inbreeding coefficients - The correlation of genes within individuals (F or F_{IT}; the overall inbreeding coefficient), or the correlation of genes within individuals within populations (F_{IS}; the within-population inbreeding coefficient). F_{IS} is also known as the fixation index. Both F_{IS} and F_{IT} are measures of deviation from Hardy-Weinberg proportions; positive values indicate a deficiency of heterozygotes whereas negative values indicate an excess of heterozygotes.

Incomplete block design – A design with a blocking structure where the number of plots within a block is less than the total number of seedlots or treatments.

Isozyme - Any of more than one form of the same enzyme encoded by different gene loci.

Land race – A population of plants, usually exotic, that has become adapted to a specific environment.

Locus - A specific site on a chromosome, usually of a gene or other marker (plural form; **Loci**).

Meristem – A plant tissue capable of undergoing mitosis and so giving rise to new cells and tissues.

Microsatellite - A subset of variable number tandem repeats characterised by very short tandem repeats with a high rate of variation in copy number among individuals. These loci tend to be randomly distributed throughout the genome and are subject to replication slippage that leads to length variation.

Mixed-effects model – A model in which there are both fixed and random effects.

Monomeric - Functional enzyme composed of a single polypeptide.

Monomorphic - Population in which all the individuals have the same genotype.

Multiple-tree plot – An experimental plot consisting of more than one tree, either as a line plot or a two-dimensional arrangement.
Natural range – The geographical and elevational limits within which an organism occurs naturally.
Neutrality - Free from the effects of selection.
Non-contiguous plots – When the trees that form a plot are not planted together but are scattered at random throughout a replicate.
Null allele - An allele that produces either no protein product or a non-functional protein product.
Nurse - A tree, group, or crop of trees, shrubs or other plants, used to nurture, improve survival, or improve the form of a more desirable tree of crop when young by protecting it from frost, insolation, wind, or insect attack.
Panmixis - Unrestricted interbreeding.
PCR – see Polymerase chain reaction.
Phenology - The study of the timing of periodic phenomena such as flowering, flushing , growth cessation *etc*., especially related to seasonal changes in temperature and photoperiod.
Phenotype - The observable characteristics of an organism due to the interaction between the genotype and the environment.
Pleiotropy – The control of more than one character by a single gene.
Polypeptide - A long chain of amino acids joined by peptide bonds.
Polymerase chain reaction (PCR) - A series of thermal cycles of denaturation, annealing of primers and primer extension catalysed by a thermostable DNA polymerase, resulting in the exponential increase of the target DNA. This method is being widely applied to obtaining DNA from old material whether dried or fossil and for the rapid sequencing of particular regions of the genome.
Polymorphism – A condition in which a population possesses more than one allele at a locus.
Polyploid - An individual containing more than two sets of genes and chromosomes.
Progeny – The offspring of a particular tree or mating combination.
Protein - A polymer of amino acids joined by peptide bonds which may be comprised of two or more polypeptide chains.
Provenance – The original geographic area from which seeds or other propagules were obtained.
Random effects – Model terms where only a single parameter is estimated describing the variation between factor levels.
Randomised Complete Block (RCB) – An orthogonal design where seedlots or treatments are randomised to the plots in each replicate.
Randomly Amplified Polymorphic DNA (RAPD) - A PCR based method which utilises short random primers to identify polymorphisms in DNA.
RAPD - see randomly amplified polymorphic DNA.
RCB – see Randomised complete block.
Recessive allele - An allele that does not express itself in the presence of a dominant allele.
REML – see Residual maximum likelihood.
Residual Maximum Likelihood (REML) - A method of parameter estimation where variance components are estimated before fixed effects.
Restriction fragment length polymorphism (RFLP) - The length of a restriction fragment produced by a particular DNA segment (probe) will be changed when the distance between two adjacent restriction enzyme cleavage sites is altered - a restriction fragment length polymorphism.
RFLP - see Restriction fragment length polymorphism.
Rootstock – The root-bearing plant or plant-part, usually stem or root, onto which another plant part (scion) is grafted.
SSR - see Microsatellites.
Seedlot – A convenience term denoting a group of seeds or their offspring considered as a unit in an experiment.
Seed source – The geographic location from which seed or other propagules were harvested.
Simple Sequence Repeats (SSR) – see Microsatellites.
Single-tree plot – An experimental plot consisting of just one tree.
Split plot design – A factorial design where treatment structures are tested at different strata.
Stumping – The practice of cutting back a plant stem either at planting time, or within the early years of establishment, to stimulate a vigorous new shoot.
Sympodial – A type of branching in which an apparent main axis is made up of many lateral branches, each arising from the branch before.
Tap root – The dominant root of a seedling or tree root system that is a direct continuation of the radicle.

Thinning – A cultural treatment made to reduce stand density of trees, primarily to improve growth.

Transplant – A seedling after it has been lifted and replanted, one or more times, in the nursery to improve its development before forest planting. The type of transplant is described by two numbers indicating length of time in years, both prior to lifting and after replanting, whilst an intermediate symbol indicates any cultural practice undertaken between these two growing periods (*e.g.* 1+1 indicates one growing season prior to lifting followed by one year after replanting in the nursery, 2u2 indicates two growing seasons both prior to undercutting (u) and after lifting).

Treeshelter – An individual guard, made of various materials and to differing heights, designed to enclose a young seedling or transplant in order to protect the plant from herbivorous grazing and herbicide drift, and to provide micro-climatic benefits to the plant by providing shelter from the wind.

Unweighted pair group method with arithmetic averages (UPGMA) – A distance matrix method which uses linear distance measures, such as standard genetic distance, to construct a phylogenetic tree from molecular data.

UPGMA – see Unweighted pair group method with arithmetic averages.

Variance component – A parameter to measure the variation between factor levels for random effects.

Zymogram - The pattern on an isozyme electrophoresis gel visualised by histochemical staining.

Chapter 1 Introduction

> *Walnut is without the question the most beautiful wood on earth, ranging from the colour of honey to the rich depth of chocolate-brown, often marked with smoky swirls and streaks of pigment from dark brown to black. The grain can be perfectly straight, elegantly swept, or a festival of waves, curls, mottles and motes, sunburst and fiddleback, as intricate as an opium dream* (McIntosh 1995).

1.1 Aims and Objectives

Aims

The aims of this research project were to initiate a study of genetic variation in a range-wide collection of walnut (*Juglans regia* L.) and to establish field trials to test this material for its suitability for timber-production in southern England. The ultimate aim of the research is to inspire a resurgence of interest in growing walnut in the British forestry industry, including researchers, practitioners and potential sponsors, with a view to strengthening, by diversification, the hardwood timber industry.

Objectives

The main objectives of the research programme, addressed in turn by each of the four main chapters within this thesis, were to:

1. Undertake a range-wide collection of walnut (*Juglans regia*).
2. Investigate some silvicultural methods for the successful establishment and management of walnut forestry plantations.
3. Analyse patterns of genetic variation in the collected material by isozyme analysis, in order to:
   - **a.** Quantify intraspecific genetic diversity;
   - **b.** Correlate levels of genetic variation, as revealed by isozyme analysis, with morphological variability detected in the field trials;
   - **c.** Provide some basic information for developing conservation strategies.
4. Establish field trials of the collected material to test performance across a range of sites in southern England.

1.2 Rationale

Juglans regia is one of the ancient introductions to Britain but today there are probably fewer trees than at any time since the late sixteenth or early seventeenth centuries. Interest in walnut as a timber waned with the increasing availability of tropical hardwoods from the early

nineteenth century onwards and it has been infrequently planted since that time, particularly as a forest tree. Supplies of hardwoods such as mahogany are now, nearly 200 years later, becoming scarce. There is also an increasing awareness by European consumers that the use of tropical timbers may contribute to deforestation, resulting in a reluctance to buy them. There are therefore good reasons to believe that valuable, decorative, temperate hardwoods are likely to be in much greater demand as tropical supplies decline.

Walnut is perhaps the finest of these valuable species, and is seen as a tree that could regain the place it had centuries ago, as the provider of high quality timber on relatively short rotations. The wood is used for making quality furniture and producing highly figured veneers, usually from burrs, which are used for cabinet-making and decorative panels. At present however, *Juglans regia* is often overlooked by foresters due to its reputation as being a species which is site demanding, usually of poor form and which suffers badly from the effects of frost.

To encourage a revival of interest in walnut in Britain, a process of selecting desirable, straight stemmed and finely branched trees to suit the climatic conditions of the country would be required from suitable parts of the species' natural range. The tree phenotypes seen in Britain today are often of poor form for timber production because their likely origin is from European trees originally selected for nut production. Phenotypes for timber or nut production are generally viewed as incompatible because good phenotypes for timber (*e.g.* long and straight stemmed, finely branched) have deliberately been selected against. Short-boled, spreading and branched trees were sought for high nut productivity and ease of harvesting. Additionally, some phenotypes in Britain may originate from ancient introductions, taken from environments unsuitable for widespread introduction to the British climate.

In order to undertake a tree improvement programme of *Juglans regia* it is important that the levels of intraspecific genetic variation are considered. The species is not classified as endangered but, as a widespread species with disjunct populations, there is likely to have been a significant anthropogenic influence on the integrity of the gene pool over time. As a widely cultivated species, intraspecific genetic diversity is likely to have been significantly reduced in some areas as a result of the introduction of clonal or narrow-based genetic material. However, no research has been undertaken to study the distribution of genetic variation of *J. regia* over both its natural and introduced ranges. A suitable study of intraspecific levels of genetic variation may reveal that the integrity of the species' gene pool is indeed threatened and could be used to plan and undertake more widespread genetic collections to address future threats from environmental change and anthropogenic influences. For the tree

improvement programme, a knowledge of the distribution of genetic variation within and between different provenance collections will be an important aid in assessing the fitness of newly introduced genotypes, not just in the present but for a future increasingly seen as heralding unprecedented environmental change.

1.3 The common walnut *Juglans regia*

The *Juglandaceae* is a large family containing seven genera and approximately 60 deciduous, monoecious trees. In addition to the genus *Juglans* L. (walnuts), the family includes *Carya* Nutt. (pecans and hickories), *Pterocarya* Kunth. (wingnuts), *Platycarya* Sieb. & Zucc., *Engelhardia* Lesche. *ex* Blume, *Alfaroa* Standl., and *Oremunnea* Oerst. (Manning 1978). There are 20 species in the genus *Juglans* which are grouped in four subgenera; *Juglans* Mann., *Trachycaryon* Dode *ex* Mann., *Cardiocaryon* Dode, and *Rhysocaryon* Dode (Manning 1978). *Juglans regia* is the sole species within subgenus *Juglans*, characterised by a four-celled nut, a husk which separates from the nut at maturity, and seedlings which exhibit two rows of scale buds immediately above the cotyledons and below the spirally-arranged compound leaves (Manning 1978). *Juglans regia* has many common names, among them the Carpathian, French, Persian and Himalayan walnut, although today it is commonly known as the English or Common walnut. It is one of two walnut species more commonly grown in Britain, the other being the black walnut, *J. nigra* L. (subgenus *Rhysocaryon*; Manning 1978), which is native to eastern North America (McGranahan and Leslie 1991). All walnut species produce edible nuts but many are encased in thick hard shells, a disincentive to commercial production, so the thin shelled *J. regia* is most widely cultivated for nut production (McGranahan and Leslie 1991).

1.3.1 The origin and history of walnut in Britain

Palynological finds of pollen and macro-fossils of *Juglans* spp. have been identified across Britain, from Kirkcudbright on the west coast of Scotland, to Norfolk in the east, and Somerset and Devon in the south (Godwin 1975). Many macro-finds have been made including remains of walnuts at Roman sites in London (Roach 1985), charcoal remains at Rotherley (Godwin 1975) and remains in the sewers of mediaeval Plymouth (Dennell 1970). There is no evidence that walnuts were actually grown in Britain in that period and all pollen records point to *Juglans* appearing from 2000 years BP (before present) (Godwin 1975); therefore it is classified as an introduced species (Stace 1997).

Walnuts were widely grown in Britain by the sixteenth century but there is evidence from an Anglo-Saxon glossary that they were cultivated from as early as the eleventh century (Roach

1985). The common name for walnut over the following centuries was based on 'walsh nutte' which referred to the Roman origin of Gaul and Italy, as opposed to the native hazel nut (Roach 1985). Roach (1985) cited several accounts in the thirteenth and fourteenth centuries that refer to 'great nuts', 'walnottes' and 'Walsnotes', including the writings of Chaucer.

The earliest description of walnut silviculture came from Gerard (1597) who wrote:

> *'The Walnut tree groweth in fields neere common high waies in a fat and fruitful ground, and in orchards; it prospereth on high fruitful banks; it love not to growe in waterie places'.*

During the seventeenth century there are frequent accounts of the use of walnut for timber. Evelyn (1678) encouraged the planting of walnuts through his writings, and planted his own trees in a family estate at Godstone, reporting in his diary that many of his friends in Surrey were making a great deal of money from walnuts and that thousands had been planted locally (Roach 1985). Evelyn also promoted the more widespread use of walnut for making chairs, tables, cabinets and other furniture, reporting that the more 'vulgar' beech was commonly used but disguised as walnut by staining it with a dye made from crushed green walnut husks (Roach 1985).

During the 1800s walnut was widely planted and locally common in Surrey, Hampshire and other parts of south-east England but by the latter half of the nineteenth century its popularity was waning, probably due to the gradual increase in the availability of mahogany, introduced early in the century from the West Indies. There was still a demand for walnut timber used in gunstocks and for coach building (Roach 1985) but according to Marshall (1803) the timber was generally going out of favour:

> *'[mahogany superceded walnut] in the more elegant kinds of furniture; and beech, being raised at less expense ... and being worked with less trouble, has been found more eligible for the commoner sorts'*

The advent of the Napoleonic Wars had a great impact on the walnut plantings in Britain as the timber was in immense demand for gun and pistol stocks and, as the number of suitable trees dwindled, prices increased dramatically (Roach 1985). The high prices offered for the timber coincided with increased payments for agricultural products and this combined effect dissuaded most landowners from replanting (Roach 1985).

In Britain today, *Juglans regia* occurs as single specimens or in small groups, and in these categories the species is rated as 'common' (Edlin 1985). In the most recent census of woodlands and trees in Britain between 1979 and 1987, no instances of *J. regia* or *J. nigra*

were recorded (Locke 1987). Chard (1949) pointed out the *J. regia* was often overlooked due to its close resemblance to ash. Mitchell (1976) reported that in Britain there were no mature trees remaining with good straight boles as these were removed for timber, although a fine 27 m tall park specimen was blown down in 1975, and that the tallest remaining trees in Britain were about 21 m tall. He also reported that two trees with girths of 4.6 m were lost a few years before that time and that few trees of the same girth remained in the country.

1.3.2 Timber properties

> *'Walnut is hard and strong, stable, lightweight, shock-resistant, flexible…. It shrinks and swells less than almost any other wood. It's sweet to work, lovely to smell, delightful to handle, and takes a splendid finish.'* (McIntosh 1995).

Walnut sawnwood is used in high class and decorative joinery for furniture and tableware, and also in decorative construction work such as in churches, houses, banks and other public buildings (Hart 1991), to which it is eminently suitable due to its strength and durability. Walnut was one of the finest timbers used for aeroplane propellers in the earliest days of aviation (Savill 1991). A large furniture-making industry based on walnut exists in Italy but the high cost of the timber restricts its use in joinery, such as for door and window frames, to a relatively small market (Natale *et al*. 1993). Veneer timber, for which a requirement is usually a pattern of some kind, is selected from the very best elements of the round timber (Natale *et al*. 1993) and typically comes from the root crown or from branch and fork areas, termed 'crotch walnut' (Hart 1991). The most valuable veneer timber comes from walnut burrs that are occasionally found on large trees (Evans 1984, Savill 1991). Harvesting walnut trees nearly always involves uprooting the tree with the root ball still attached. This allows the most valuable timber, at the root crown, to be fully utilised (Hart 1991, Natale *et al*. 1993). Major markets for walnut veneer are in the furniture and the car manufacturing industries (Hart 1991). Walnut has been widely used in gunstock manufacture where it is generally regarded as the best timber because it is excellent for holding metal parts and also shock-resistant, lightweight and highly decorative (Fairley 1955).

The value of walnut sawnwood and veneer logs is substantially greater than other broadleaved species. Eastern European walnut prices in 1990 were double those of oak for both veneer and sawn timber (Berenyi *et al*. 1990). Savill (1991) quoted prices paid for walnut logs in Germany between 1978 and 1986, ranging from £90 to £1100 per m^3. There is often great variability in prices paid for walnut timber due to huge variations in quality, difficulties with supply and poor marketing strategies (Natale *et al*. 1993). Up-to-date prices paid for walnut in Britain are difficult to come by as the timber is sold on a tree-by-tree basis, with prices

varying according to size and quality (Goodwin[1], pers. comm.). Recent (April 2000) timber prices in Italy, which consumes 50 % of the walnut veneer sold in Europe (Carizi and Molteni[2], pers. comm.), start at £700 /m^3 for minimum veneer grades (minimum length 2.2 m/ diameter 40 cm), whilst prices for good veneer grades range between £1000 and £1100/m^3 ([3]) but can reach much higher values for the best veneer grades (Petillo[4], pers. comm.). Decreasing volumes of walnut timber available on the European market, combined with an increasing demand for valuable wood products in general, are likely to inflate prices in the future (Rotach 1998).

1.3.3 Current status of walnut silviculture and future potential

Juglans regia is a species well known to be exacting in its site requirements (Steven 1927, Chard 1949, Evans 1984, Istvan and Tibor 1990, Savill 1991). Steven (1927) suggested latitudinal limits of between 44^o and 54^o N for successful cultivation, which in Britain limits cultivation to the south of England. The cooler and moister summer climate of northern Britain is less suitable for walnut cultivation than southern England because walnuts thrive in regions with warm summers (Evans 1984). The major problem with growing walnuts in Britain, and in fact throughout its natural and introduced ranges, is damage by frosts, both in late spring, when young shoots and flowers are very susceptible, and early autumn (Evans 1984, Savill 1991). The importance of selecting for late leaf flushing in breeding strategies for walnut cannot be overstated and potentially may be achievable, as flushing is highly heritable (h^2 = 0.96; Hansche *et al.* 1972).

Given the potential value of producing home-grown walnut timber, and the difficulties associated with its silviculture, it is perhaps surprising that such little research has been conducted on developing better methods of walnut silviculture or on testing improved material for walnut timber production in Britain. Between 1986 and 1987, four experiments were established by the Forestry Commission to assess the growth and survival of two provenances of *Juglans regia* (Hungarian and English) and six of *J. nigra* (Kerr 1993). Four sites were used in the south of England, the most northerly being situated in Northamptonshire. The conclusions were that the site is of greater importance than provenance in terms of survival and height increment, and that on the range of sites used, *J. nigra* performed better than *J. regia*. This experiment remains the only attempt to study the

[1] Peter Goodwin, Titchmarsh and Goodwin Cabinet Makers, Ipswich, England.

[2] Carizi and Molteni, Veneer manufacturers, Pesaro, Italy.

[3] http://www.arsia.toscana.it/legno/prezzi

[4] Dr. Genaro Petillo, Walnut srl, Napoli, Italy.

potential for growing walnut in Britain using selected material. It is the author's opinion that this research, based on an inadequate range of material, has done little to boost the British forester's confidence in the species. Walnut, particularly *J. regia*, is seen by the Forestry Commission as a minor species of little economic importance on a national scale, as is evident from its exclusion from current breeding strategies (Forestry Commission 1994).

Provenance collections of *Juglans regia* were initiated in the early 1980s in Switzerland and include 20 sources from wild populations in India, Pakistan, Nepal and Bhutan (Rotach 1998). Several provenances, particularly material collected from Bhutan, were found not to be sufficiently frost-resistant. After nine growing seasons, material from Pakistan and Kashmir was showing good potential with 35 % of genotypes considered of suitable quality for furniture production and 35 % suitable for veneer quality production (Rotach 1998). A European Commission-funded joint research programme (Proposal PL96-1887 in FAIR III) focused on the production of high quality wood for furniture, by walnut species (*J. regia*, *J. nigra* L. and their hybrids) involving breeding programmes and the development of new technologies and markets (Jay-Allemand, pers. comm.[5]). The goals of this programme are to increase the number and the quality of walnut trees planted in Europe and to standardise the production of walnut timber according to their utilisation. The UK has no formal links with this programme.

The following chapters address the main objectives of the research (Section 1.1), which together constitute the essential elements of a new breeding programme, aimed at improving the suitability of *Juglans regia* as a timber-producing species in Britain. Chapter 2 describes the distribution of *J. regia* and details the collections of genetic material on which much of this work was reliant. Chapter 3 is concerned with silvicultural research undertaken to provide much needed direction towards the successful cultivation and establishment of walnut. Chapter 4 is devoted to the study of genetic variation, which was undertaken throughout the period of this programme, whilst Chapter 5 reports on the provenance/progeny trials established during the final year of the research programme.

[5] Co-ordinator, Walnut B.R.A.I.N.S. (Basic Research for Agroforestry and Industry: Network and Standards). www.juglans.org.

Chapter 2 Genotype collections

2.1 Introduction

A range-wide collection of *Juglans regia* material was gathered during the autumn of 1997 to facilitate the project's joint main aims of selecting new and improved walnut phenotypes for timber production in the UK, and analysing genetic variation in the species. This chapter describes the distribution of the species, the criteria adopted for the selection of populations and the sampling techniques employed. Section 2.2 is devoted to describing the natural and extensive walnut forests of Kyrgyzstan, at the heart of the species' natural range, and presents the results of a seed collecting expedition that was undertaken to this remote country in the autumn of 1997.

2.1.1 The distribution of *Juglans regia*

The natural range of *Juglans regia* is confined to the Asian continent (Figure 2.1) extending across twenty one modern political boundaries: from Turkey, Azerbaijan, Armenia, Russia, Georgia and Iraq (Kurdistan) in the west, across the northern lands of Iran and Afghanistan and the heart of central Asia in the newly independent states of Kazakhstan, Turkmenistan, Uzbekistan, Kyrgyzstan, Tajikistan and their giant neighbour China in the Xinjiang Autonomous Region, formerly 'Chinese Turkestan', extending further south in a narrowing range nestling in the mountains of Pakistan, northern India and Nepal, and finally reaching its eastern extent in Bangladesh, Myanmar (Burma), Bhutan and southern China (Nekrassowa 1927, Schmucker 1942, Berg 1950, Browicz 1976, Davis 1982, Puri *et al*. 1983, Komarov 1985). Jalas and Suominen (1976) and Tutin *et al*. (1993) believe that *J. regia* may be native to Greece and elsewhere in the Balkan Peninsula.

Juglans regia has a range distinct from other *Juglans* species, only narrowly bordering the western extent of *J. cathayensis* Dode in eastern Asia (Schmucker 1942, McGranahan and Leslie 1991). *Juglans regia* grows naturally in the mountainous regions of Asia at a wide range of altitudes. In the Anatolian region of Turkey, it is found growing naturally between 1150 and 1250 m above sea level (a.s.l.) (Akca and Sen 1994). In the northern forests of Iraq, in the Kopet Dagh mountain range, Atefi (1990) reported that natural stands of *J. regia* grow from sea level to 1500 m a.s.l. and it also occurs in the western forests between Marivan and Sanandaj. Berg (1950) stated that *J. regia* is found in the wetter gorges of the central Kopet Dagh but only up to 1200 m a.s.l. Near Darvaza in Turkmenistan, Berg (1950) reported that individual walnut trees could be found at elevations up to 2300 m a.s.l., and in central Tajikistan the species occurs as continuous forest up to elevations of 2200 m a.s.l.

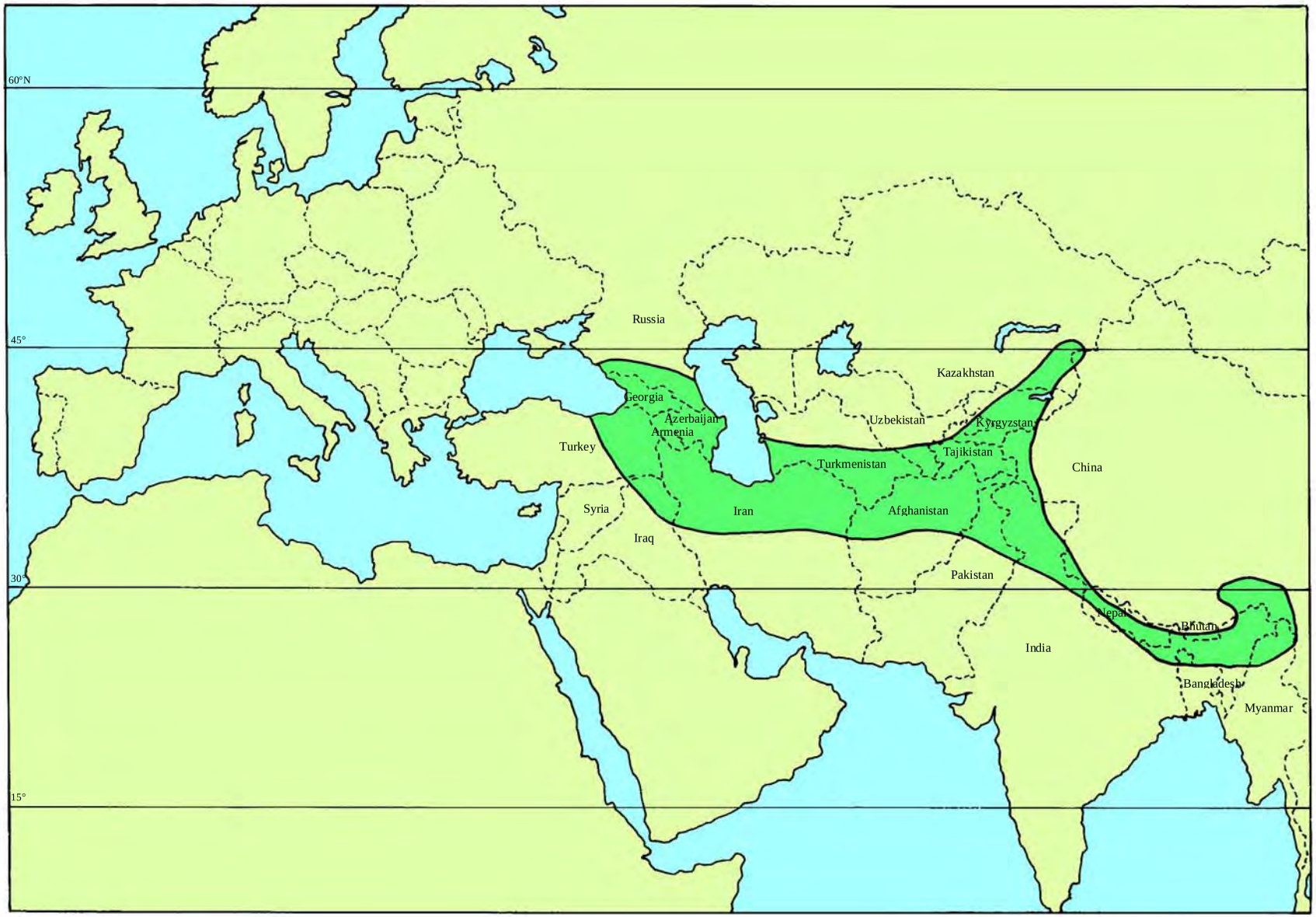


Figure 2.1 The natural range of walnut *Juglans regia* (shown in dark green). Based on Schmucker (1942) and McGranahan and Leslie (1991).

Krassilov (1995) reported that walnut forests are characteristic of the Pamir-Alay range, the Kopet Dagh and the western Tian Shan at elevations of 1000 to 2300 m a.s.l. East of these areas it has a scattered distribution, following the Himalayas across the northern boundary of India and Nepal. *Juglans regia* is found between 1900 and 2400 m a.s.l. in the wetter mountains of northern Afghanistan, namely in the Nuristan Safed Koh and the northern Sulaiman ranges (Puri *et al.* 1983).

The natural distribution of *Juglans regia* is generally governed by a continental climate. The Anatolian region in Turkey, which is at the western extent of its natural range (Barut 1996), has a wide ranging climate from the mild and humid Black Sea region, to the subtropical climate of the coastal regions and the continental climate of hot dry summers and cool winters inland to the south east. Today, walnuts are found growing across all these regions but the extent of native forest is largely limited to central, north and north-east Turkey (Davis 1982). Near the town of Gürun in central Anatolia where the average annual temperature is 9.5 ^{o}C, there are reported to be more than 10,000 naturally occurring walnut trees (Akca and Sen 1994). In Turkey, *J. regia* is found in mixed deciduous forest, mostly of *Quercus* spp. on calcareous rocky slopes and in valleys of alluvial soil with a high gravel content (Davis 1982). In the Himalayan mountains, it grows in broadleaved forests consisting of *Quercus dilatata* P.M. Kern and *Q. semecarpifolia* Caval-Sm. in areas with heavy summer rainfall in northern Afghanistan, Pakistan and north-west India (Puri *et al.* 1983). *Juglans regia* also occurs amongst the coniferous forests of the western Himalayas in mixed groups of broadleaves, including *Acer caesium* Kosterm. and *Ulmus wallichiana* L. amongst *Abies* spp. and *Picea* spp. The broadleaved community is particularly well developed in the Kashmir Valley.

There are indications that *Juglans regia* was present in China 6000 to 7000 years BP. Carbonized walnut shells have been found in the ruins of Cishan Hebei and been dated to 7000 years BP, and walnut pollen has been identified in regions of primitive settlements and dated to 6000 years BP (Guozhen and Weichang 1990, Xi 1990).

There is some disagreement among authors as to whether *Juglans regia* is native or introduced in Europe. Its widespread cultivation has only served to confound the evidence for the extent of native and introduced populations. Jalas and Suominen (1976) and Tutin *et al.* (1993) stated that *J. regia* is native to areas of the Balkan Peninsula, especially Greece, the former Yugoslavia, Romania and Bulgaria. Jalas and Suominen (1976) also reported that the populations present in southern Italy, Sicily and north-east Austria are of ‘unknown or

uncertain' origin. Huntley and Birks (1983) suggested that evidence for the claim that *J. regia* is native to the Balkans region is based on its association with the forest region in which it is an important element in the canopy. There it cohabits with *Castanea sativa* Mill. and other restricted species such as *Platanus orientalis* L. and *Corylus colurna* L. and it is to this forest type that is reported to be native.

In the Holocene, the earliest reappearance of *Juglans* pollen was approximately 5000 years BP (Beug 1962). By 4000 years BP, locally well established populations were present in the southern Alps of Italy and the Balkans and these have been linked to vegetational disturbances by Neolithic man (Beug 1962). Huntley and Birks (1983) proposed that the expansion may not have been deliberate by man but an opportunistic expansion by the species. This in turn suggests that there were refugial populations present in these areas during the Würm glaciation, which slowly migrated westwards in the early Holocene (Beug 1962, Filipovitch 1977).

The dispersal of the *Juglans* population between 4000 and 2000 years BP was slow and this may reflect the slow natural dispersion rate of the species, due to the weight and palatability of the nuts. Beug (1962), Filipovitch (1977), Bottema (1980), Zohary and Hopf (1993) and others point to the late return of walnut pollen to most of Europe, from 2000 years BP onwards, as an indication that *J. regia* was reintroduced to Europe from the east by man and not as a result of natural post-glacial expansion. However from 2000 years BP the species expanded rapidly at up to 400 m yr^{-1} (Huntley and Birks 1983) and many authors have linked this to the influence of the Romans and Greeks (Beug 1962, Davis 1982). Huntley and Birks (1983) also pointed out that the climate of the Roman era was more favourable than that of the present day and this may have led to increased rates of natural dispersion.

The high pollen values present at 1000 years BP reflect the widespread cultivation and planting of walnut across most of Europe (Huntley and Birks 1983). Indeed, Zohary and Hopf (1993) proposed that the populations of walnuts now growing in central Europe and the Balkans, represent feral progeny of the original cultivated walnuts introduced from the Bronze Age onwards and that north-east Turkey, the Caucasus and north Iran are the most likely origins of domesticated walnuts.

2.1.2 Acquisition of seeds

Walnut seeds were sought from as many locations as possible, both within the natural range and in the current range of the species. Two approaches were taken in gathering material.

Firstly, the author undertook a seed collecting expedition to Kyrgyzstan, within the natural range of *Juglans regia*, the details of which are covered in Section 2.2. Secondly, scientists and tree breeders within national forest institutes and similar organisations were approached by letter and asked whether seeds could be supplied for research (Section 2.3).

The sample size necessary to sample genetic variation thoroughly in a population can be calculated, especially in respect to sampling rare alleles for strategic conservation, but requires a prior knowledge of allele frequencies in the population genome (Krusche and Geburek 1991). The values of the genetic diversity parameters of the populations could not be known prior to sampling, and although theoretical computation methods exist (Brown and Marshall 1995), the main aim of this project was not specifically to search for rare alleles. FAO (1995) guidelines suggested that for a widespread species, such as walnut, a sufficient sampling of the gene pool for provenance/progeny trials usually involves 10-20 individuals per provenance and that the distance between individuals sampled within a stand should be not less than the distance of normal seed dispersal. The aim of defining these distances is to minimise the likelihood of sampling related individuals (FAO 1995). Walnut seed is generally gravity dispersed, although the possibility of animal dispersal cannot be ruled out (Chard 1949, Davis 1982), whilst maximum pollen dispersal has been reported at 300 m (Popov[1], pers. comm.). For sampling in Kyrgyzstan a minimum of 50 m between individuals was selected which was deemed to reduce the likelihood of sampling maternally related trees, and 20 to 30 individuals were the target number of parent trees per provenance. For the seed collections from the other countries, where there was less control over the collection method, some broad prescriptions were outlined to the collectors. They were asked to supply additional information on the environment from which the parent trees were situated (latitude, longitude and altitude), the characteristics of the parent trees themselves (height, *dbh*, and age) and to maintain maternal identity where possible.

2.2 Provenance collections from Kyrgyzstan

The genetic resources of forest trees are usually located in primeval or ancient forests (Frankel *et al.* 1995). The mountainous country of Kyrgyzstan, containing a substantial area of natural walnut forest, was the main focus for seed collecting and was visited by the author in an expedition to sample populations of natural forest genotypes. Kyrgyzstan is situated at the northern limit of the natural range of the species (Schmucker 1942, McGranahan and

[1] Sergei Popov, Institute of Forest and Walnut Breeding, Kyrgyzstan.

Leslie 1991) (Figure 2.1). In provenance selection, the movement of seed from sources of origins that are very different from the introduction location can be problematic if high altitude or high latitude sources are moved to low altitudes or low latitudes, or indeed the reverse (Zobel and Talbert 1984). However, high altitude sources from low latitudes can often be introduced successfully to low altitude locations at high latitudes (Zobel and Talbert 1984). Therefore the seed sources from Kyrgyzstan, although lying 10° south of the UK, provided a possible source for successful introduction due to the altitudinal difference between the two locations.

The collections from Kyrgyzstan are referred to as provenances although the term 'provenance' is often used ambiguously in forestry literature. Here, provenance means:

> *a seed collection unit composed of a community of potentially interbreeding trees in an area subject to uniform environmental conditions.*

This definition was adopted after consideration of the review by Turnbull and Griffin (1986) and is strictly only applicable to the Kyrgyz material. The concept of genetic constitution as a criterion for defining a provenance is not adopted here although the uniformity within a provenance, or the differentiation between provenances, has been proposed by some authors (Turnbull and Griffin 1986). The concept of 'populations', as defined by genetic analysis, is one of the main aims of this project (Chapter 4).

2.2.1 The walnut forests of Kyrgyzstan

Kyrgyzstan lies at the mountainous heart of Central Asia, a floristically diverse region containing about 7000 vascular plant species (Krassilov 1995). Five thousand of these occur in the mountain regions (Krassilov 1995), including *Juglans regia* where it forms extensive forests. The natural walnut-fruit forests in Kyrgyzstan have been identified by the International Union for the Conservation of Nature and Natural Resources (IUCN), as an extremely valuable reserve of rich genetic diversity (Krassilov 1995). The forests have a distinct species composition of *J. regia* and other smaller trees and shrubs including *Malus* spp., *Pyrus* spp., *Prunus* spp., *Pistacia vera* L., *Crataegus* spp., *Amygdalus* spp., *Juniperus* spp., *Ribes* spp. and *Rosa* spp. The large tracts of walnut forests are situated in two main areas in Kyrgyzstan stretching from east to west; at Arslanbob-Kurgat and Khozha-Aty, on the slopes of the Fergana and Chatkal mountain ranges respectively. In the Chatkal range two distinct floras converge, the northern one is represented by *Abies* and *Picea* (including an indigenous spruce, *Picea schrenkiana* Fisch. & Mey.), and the southern flora of *J. regia* and other broadleaved tree and shrub species. This area was designated as the Sary-Chalek National Park in 1945, named after a high altitude lake in the territory, and supports 969

higher plant species (52 endemic to the Tian Shan) and over 40 mammals and 160 birds (Bukshtinov *et al.* 1981). The walnut-fruit forests in both mountain ranges occur between 800 and 2100 m a.s.l., although 82 % of walnut stands are located between 1400 and 1750 m a.s.l. (Kolov 1998). According to survey data collected between 1966 and 1968, pure walnut stands cover 25,600 ha, which is approximately 11 % of the 230,700 ha of the natural forest area (Musuraliev 1998).

The origin of the walnut forests has been a subject for debate among botanists and other scientists in Central Asia because they are isolated in the mountain regions by surrounding semi-desert areas. The forests were once believed to have originated from the Tertiary (Ashimov 1998) but the current consensus is that the forests of southern Kyrgyzstan originated at the end of the Pleistocene (Vyhodtsev 1970), supported by some pollen evidence from the Quaternary (Grishina 1968). The climatic conditions that support the forest ecosystem are created by the topography of the region, where the orientation and length of the mountain ridge-tops reduce the intrusion of the cold northerly air streams (Kolov 1998). The microclimate produced is characterised by moderate temperatures in the summer, mild winters, and abundant precipitation in the spring (Kolov 1998). Rainfall is carried by western air currents (Ponomarenko 1976) and is quite evenly distributed throughout the year, although July and August are the driest months. In the Fergana range the average annual rainfall is 1090 mm and the summer season is warm, with an average July temperature of 20.5 ^{o}C whilst winters are short and mild with an average January temperature of –3.1 ^{o}C, although minimum temperatures can drop to -24 ^{o}C (Anon. 1966, Anon. 1969). In contrast to the Fergana range, the Chatkal range is characterised by colder and more humid conditions which, in combination with less favourable soil conditions, results in a lower tree-line (Sherbinina 1998).

Juglans regia is capable of growing on unstable slopes, developing strong widespread roots in the upper soil layers (Ma and Xi 1990). The walnut forests therefore play an important role in protecting the steep slopes from erosion and subsidence (Zapryagaeva 1964), a fact borne out by frequent mud-slides in denuded forest areas, such as in the Kyzyl-Ungur river valley in 1987 (Sherbinina 1998). All the rivers that irrigate the main cotton-growing areas in the Fergana valley rise in the walnut-clad slopes of the Fergana mountain range. The walnut forests therefore play an important role in the region’s hydrological cycle (Matveev 1984).

Few areas of the walnut-fruit forests remain untouched and in certain locations walnut timber was extracted in large quantities until the early twentieth century, by which time substantial

areas had been deforested (Musuraliev 1998). Between 1896 and 1926, 500 tonnes of walnut burrs were exported to France and England for an equivalent price to weight value as silver (Vyhodtsev 1970). Many of the clear-felled areas were subsequently used for arable production or for the creation of new settlements (Musuraliev 1998). Since 1950, forest inventories have indicated a considerable decrease in the density of walnut stands which are also becoming increasingly senile, to the point where by 1998, 47 % of the forest area is comprised of over-mature trees, 31 % mature trees, 15 % immature trees and only 6 % trees under two years old (Venglovsky 1998). However, there remains some 2.08 million m^3 of standing timber, averaging 75 m^3 ha^{-1} over the whole forested area but 249 m^3 ha^{-1} in mature and over-mature stands (Kolov 1998). Between 1981 and 1996, 30,000 ha of forest in the fruit-forest reserve were cleared and 24,000 m^3 of timber extracted (Musuraliev 1998). In an average decade, 5,774 m^3 of walnut timber are harvested by local leskhozes (forestry farms) and processed into veneer blocks for the local furniture industry (Musuraliev 1998).

In addition to some unfavourable environmental factors (including soil moisture deficits in late summer, pests and diseases, and late spring/early autumn frosts), unregulated and intensive human activity in the forest has led to an increasing concern regarding the condition of the walnut-fruit forests (Kolov 1998). Fifty years of research has indicated that the average nut crop varies from 15 to 25 kg ha^{-1} (approximately 1500 –2500 seeds), and that an individual tree's seed production typically peaks at 130 years and continues until the tree is 300 to 400 years old (Chebotarev 1970, Venglovsky 1998). However, the nuts are efficiently harvested by the local populace of some 50,000 people who live within the forest, for their own consumption or for selling in local markets (Musuraliev 1998). The effect of these factors, in combination with hay-making in the forest clearings and with substantial overgrazing by some 49,000 free-ranging livestock (Musuraliev 1998), has made natural regeneration virtually non-existent except in the most inaccessible areas. The principal method of regeneration observed in many areas of the forest over the last 150 years (Gan and Venglovsky 1997) is natural regeneration by coppice growth, with multiple stems arising from the butts of old trees with fallen crowns.

2.2.2 Provenance locations and properties

The expedition to collect seed was undertaken by the author with the assistance of Dr. Peter Savill[2] between September 21st to October 11th 1997. This is the time at which walnut seeds mature in this region and hence the optimum time for seed collection (Venglovsky[3], pers.

[2] Dr Peter Savill, Silviculturist, Oxford Forestry Institute, University of Oxford.

[3] Bronislav Venglovsky, Director, Kyrgyzstan Forest Institute.

comm.). Planning for the expedition took place over approximately nine months and was aided greatly by the Kyrgyzstan Forest Institute and the Swiss non-governmental organisation LES-INTERCOOPERATION. Two main walnut forest areas, located on the slopes of the Fergana and Chatkal mountain ranges, were identified as target sample areas.

The provenance locations were selected on a number of criteria, the most important of which was that provenances should be separated sufficiently by distance or physical terrain to reduce the likelihood of one provenance commonly interbreeding with another. Samples were collected across a latitudinal gradient whilst specific locations for sampling were selected according to altitude and aspect. The aim was to sample representative provenances across an altitudinal range of walnut whilst also sampling from stands located in different topographical conditions.

The main sampling area during the expedition was in the Fergana Mountains with an additional area selected from the Chatkal Mountains (Figure 2.2). A total of eleven provenances was collected; nine from the Fergana range, located on the southern slopes and foothills of a lateral ridge of the mountain range, and two from the Chatkal range, sampled from within the Sary-Chalek Biosphere Reserve. All but one of the provenances were collected from natural forests, the exception being K1 which was a small plantation reportedly planted in the 1940s using stock raised from a local seed source (Popov, pers. comm.).

A hand held Global Positioning System (GPS) was used to register the location of the centre of the sampled provenance; the latitude and longitude co-ordinates being given in degrees, minutes and decimal seconds to an accuracy of 30 m (Table 2.1). The GPS readout, using four or more satellites, was used to give altitudinal elevations (+/- 30 m). The latitude and longitude co-ordinates generated from the GPS were used to mark the positions of the provenance locations accurately and are shown in Figure 2.2. The closest neighbouring provenances (K1, K3, K4 and K8), which were one to two kilometres apart, were located in different valleys and, as such, regular interbreeding between the different provenances could effectively be ruled out. Travel within the region was difficult and time consuming. For example to travel by four-wheel drive from the base (located near K2) to K7 took over two hours. To travel from the Ak-Terek region in the Fergana mountains to the Sary-Chalek Biosphere Reserve (K10-11) took eight hours in a four-wheel drive vehicle. These difficulties prevented the collection of additional provenances over a wider area.

Table 2.1 Site descriptors for the provenance locations in Kyrgyzstan.

Provenance	**Region**	**Mountain range**	**Longitude**[1] (° East)	**Latitude**[1] (° North)	**Altitude** (m)	**Aspect** (°)	**Slope angle** (°)	**Soil texture**	**Basal Area** (m²/ha)	
									Walnut	**All spp.**[2]
K1	Ak-Terek	Fergana	72.49.9	41.17.5	1700	220	5	silt loam	19	19
K2	Ak-Terek	Fergana	72.50.1	41.15.5	1390	30	32	silt loam	18	18
K3	Ak-Terek	Fergana	72.49.1	41.17.8	1860	180	26	silt loam	17	23
K4	Sharap	Fergana	72.51.7	41.16.4	1620	0	22	sandy loam	16	19
K5	Yaradar	Fergana	72.59.0	41.19.2	1260	180	10	silt loam	20	20
K6	Shaidan	Fergana	72.47.7	41.16.8	1590	60	20	clay loam	17	23
K7	Kyzyl-Ungur	Fergana	73.05.7	41.23.1	1400	330	27	sandy loam	14	17
K8	Katar-Yangak	Fergana	72.50.8	41.18.7	1900	160	10	silt loam	11	23
K9	Kyok-Sarau	Fergana	72.53.1	41.18.0	1830	40	8	silt loam	14	16
K10	Kyr-Sai	Chatkal	71.57.4	41.50.6	1320	60	9	sandy loam	13	13
K11	Ters-Kolt	Chatkal	71.56.6	41.49.5	1440	0	10	sandy loam	12	13
MEAN					1460		16		16	19

Longitude and latitude [1] readings are given in degrees, minutes and decimal seconds.
All species [2] refers to the basal area of all species; *Juglans regia* and understorey species (no other canopy species were found at any of the sites).

Environmental details of each sample location were measured and recorded. Slope aspect was measured using a compass. Slope angle was measured with a Suunto clinometer to an accuracy of one degree and the mean of a minimum of five readings was used to classify the site. Soil texture was analysed by a simple field hand-texturing technique that relies on the varying malleability of different soil types when moistened (Trudgill 1989).

Table 2.1 summarises the provenance locations and the site descriptors for all the measured parameters. The altitude of the provenances varied from 1260 to 1900 m above sea level whilst trees were sampled from a wide variety of slopes with different aspects. Seven out of the eleven provenances were collected from slopes with a northerly aspect whilst none was sampled from west or east aspects (Table 2.1). The soil textures of the provenances were all of a loamy type but varied from clay loam to sandy loam (Table 2.1). In addition to the features described above, a brief verbal description of the ground vegetation and general environmental conditions for each provenance was recorded (Appendix II, Table II.2). The sites varied from steep ravines, to ridge tops, valley sides and to gentle slopes, all of which had different understorey densities and ground vegetation composition. Only the most severe slopes of 25° or more, escaped heavy grazing by sheep, horses and/or cattle. Consequently, there was a virtually no natural regeneration in most of the visited stands.

The forests consisted entirely of walnut as the canopy species (Plate 2.1). Two individual trees in different locations, both *Fraxinus* spp., were the only records of other canopy tree species. This is supported by the basal area measurements (Table 2.1) which indicate that walnut was commonly very dominant. The difference in the figures for 'total' and 'walnut' basal areas in Table 2.1 therefore represents understorey species only: usually *Acer turkestanicum* Pax., *Crataegus* spp., *Prunus* spp. and *Malus* spp. The typical density of walnut trees was estimated to be 80 to 100 ha^{-1}.

Once a suitable provenance site had been located according to the criteria listed above the most fundamental factor was seed availability. Identification of seed production within a stand and of individual fruiting trees was aided by the use of a pair of 8 × 30 binoculars. The female flowers of *Juglans regia* are produced from a terminal shoot on the current year's growth and the drupes were therefore usually visible on the periphery of the tree crown.

Individual trees were selected for sampling based on the presence of desirable phenotypic characters, generally indicated by a relatively branch-free and straight stem, whilst trees of varying age within the stand were also sought in order to sample diverse generations.

Plate 2.1 Looking south from a ridge top at 1860 metres altitude, on the edge of Provenance K3 at Ak-Terek, Kyrgyzstan. Walnut-fruit forest extends as far as the eye can see. The canopy consists almost entirely of *Juglans regia* whilst the understorey is a mixture of shrubs; *Acer* spp., *Crataegus* spp., *Prunus* spp. and *Malus* spp.

There was no attempt to sample trees randomly. In certain areas where superior phenotypes were not present, the best available ones were selected. Trees with a poorer phenotype were therefore not completely excluded from the sampling strategy. This was advantageous because genotypes that may have been adversely affected by the environment, thereby producing poor phenotypes, were sampled: *e.g.* coppiced trees were included in the sampling even though their potential phenotypic structure as single-stemmed trees could not be directly assessed.

The relative position of each of the sampled trees within a provenance was recorded on a sketch map. Following genetic analysis, this may allow the genetic diversity of individuals to be correlated with their position within the provenance: *i.e.* whether located as an outlier, on the periphery or centrally in the provenance, and the identity of nearest sampled neighbour(s).

Once an individual tree had been identified as suitable, five to ten seeds were collected from each tree, which were preferably removed directly from the crown to ensure parentage, as recommended by FAO (1995). The seeds were collected from all parts of the crown so that there was a greater potential for different pollen sources being sampled, as suggested by Brown and Marshall (1995). Smith (1995) discussed various methods of removing seeds from a tree crown for sampling genetic variation. The remoteness of the region however ruled out many of these options and so a simple throwing-stick (a 45 cm length of freshly cut walnut branch wood) was effectively used up to heights of 34 m. In some cases where seeds were scarce, nuts were collected directly from the ground below the canopy, in which case careful study of nut morphology (including groove patterns on the nut surface) was used to ensure parentage.

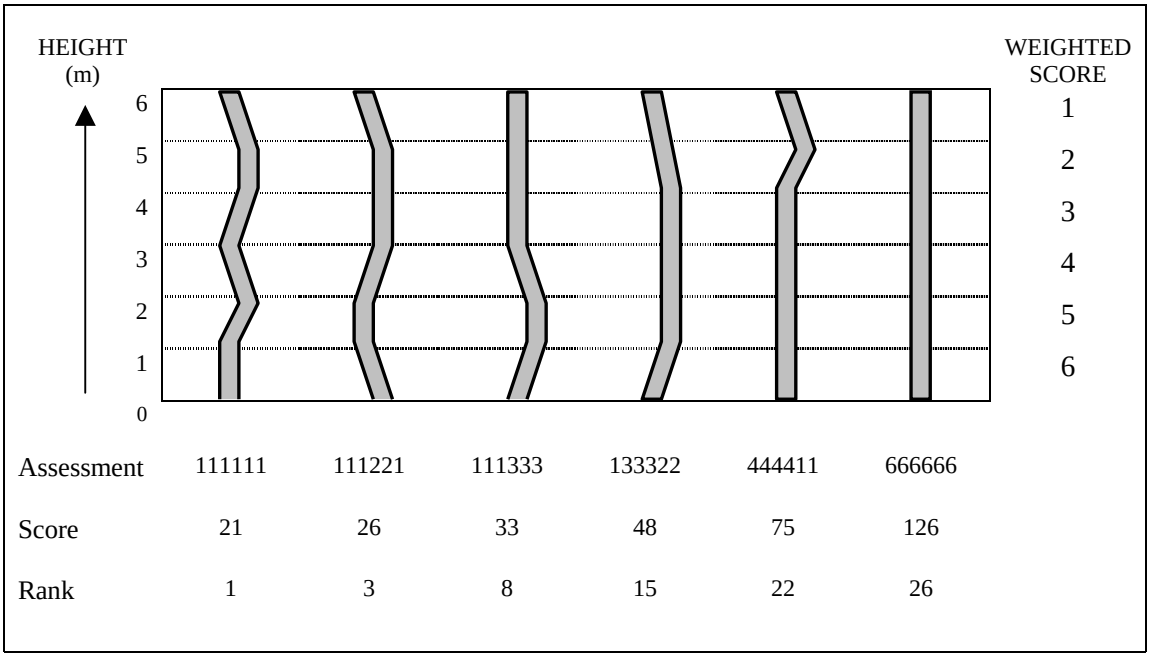
The ripeness of the drupe is critical when collecting the seed, which was ideally when the green husk had turned black and was just starting to split. If the fruit had been too long on the ground or on the tree, the husk would become putrid and increasingly difficult to remove as it hardened, as described by Gordon and Rowe (1982). Once the drupes were collected, all green parts of the husk were removed as these can provide sites for fungal infection (Crawford 1996). Seed viability was assessed directly in the field by estimating the relative weight of a seed. It was found that with some experience the identification of rotten, diseased or undeveloped seeds could be successfully judged by ‘feeling’ the weight of the seed in one’s hand. Each provenance sample, containing several seeds from numerous sampled trees required clear identification to record the parentage of progeny. An effective method was to write the parent tree’s identification number directly onto the walnut shell, using a permanent

marker pen. On the same day, following a collecting session, the seeds were removed from the collecting bag and spread onto flat shelves, to dry to a lower moisture content, out of direct sunlight at normal air temperature (> 0 ^{o}C and < 30 ^{o}C), in a well aired room for about five days, as recommended by Gordon and Rowe (1982).

2.2.3 Parent tree and seed details

Eleven descriptors for the parent (maternal) trees were developed and incorporated into a single recording sheet for each provenance. The total tree height and the height to the first branch were calculated from measurements taken using the percentage scale of a clinometer. For those parent trees that were of coppice origin, only the dominant stem of the stool was measured. Diameter of the stem at breast height (1.3m; *dbh*) was measured directly with a diameter tape (Plate 2.2). Accuracy was maintained by ensuring that the tape did not pass around burrs or other protrusions whilst stem buttresses were avoided by measuring the stem at a greater height if necessary. Branch angle was scored according to a simple three point system, based partly on the walnut timber descriptors of Ducci and Veracini (1990), where '0' represented branches with approximate angles of 90^{o} to 60 ^{o} from the stem, '1' equalled 60^{o} to 45^{o} and '2' represented 45^{o} or less.

Stem straightness was classified according to a simple but very effective method developed for tropical pines by Barnes and Gibson (1986) (Figure 2.3). The method is dependent on the potential timber value of a stem and relies on the classification of a predetermined section of



Assessment	111111	111221	111333	133322	444411	666666
Score	21	26	33	48	75	126
Rank	1	3	8	15	22	26

Figure 2.3 Stem straightness assessments for a range of stem qualities, redrawn from Barnes and Gibson (1986).

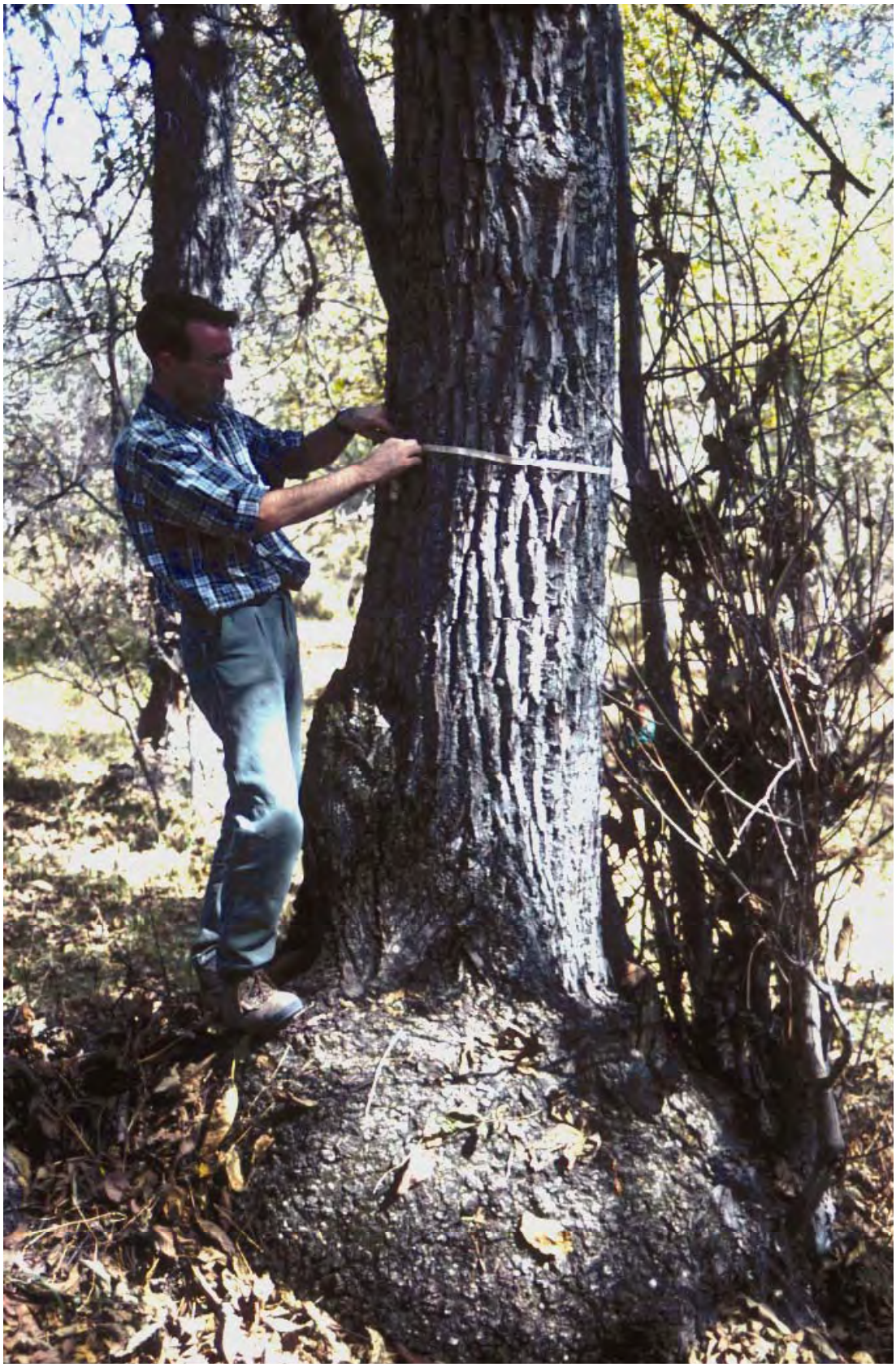


Plate 2.2 Measuring stem diameter at breast height (*dbh*) of a walnut at Kyok-Sarai (K9), Kyrgyzstan. The author is standing on a substantial root burr.

stem as being straight or bent. In order to score the trees accurately by their true value, a view of the stem from at least two different angles, 90^{o} apart, was necessary because a stem may appear to be straight from one view if a bend is present perpendicular to that view. A weighted score was then applied so that the lower straight sections of stem, having a greater potential economic value, were scored more highly than straight sections at a greater height up the stem (Figure 2.3). The first 6 m of each parent tree were scored as this covered the standard timber lengths for walnut, of two or more metres of straight timber (Carizi and Molteni[4], pers. comm.). The scores were then ranked with values ranging from 1 to 26, the lowest represented 6 m of stem without any straightness, the highest represented 6 m of continuous straight stem (a worked example is given in Appendix II, Table II.1).

Crown diameter of the parent trees was measured in seven of the 11 provenances although the steep terrain on some sites, as well as time constraints, made measurement impractical at four of the locations. Two crown diameter measurements, at right angles to one another, were taken for each parent tree and the mean recorded. Measurements were taken by two operators who stood at opposite sides of the crown with a 25 metre tape, ensuring that the tape passed below the centre of the crown. The operators ensured they were directly below the crown edge by sighting vertically with a clinometer aimed at 90° towards the maximum outer extent of the crown.

The presence or absence of burrs on the stems, or root collars, of parent trees was recorded and coppiced trees were simply recorded as such. Additionally, leaflet morphology of each parent tree was scored according to descriptors developed by the International Plant Genetic Resources Institute (IPGRI 1994). The seeds were measured using vernier callipers, in two dimensions; for face width and length. Seed morphology was classified according to IPGRI (1994) seed descriptors (Table 2.3, p. 28). Mean seed weight for each provenance was measured later, after the seed had dried to a lower moisture content in spring 1998, just prior to sowing (Section 3.1).

The basal area of the stand at each provenance location was measured at several points using a simple relascope, a ‘dendrometer’, consisting of a cross piece of known width and a distance piece of known length. The number of sampling points used varied between 8 and 12 depending on the area sampled and the heterogeneity of the stand of trees, as advised by Hamilton (1975). Measurements of both total basal area and walnut basal area were taken and are summarised in Table 2.1 (p. 18).

[4] Carizi & Molteni, Pesaro, Italy. Veneer manufacturers.

2.2.4 Results

In Kyrgyzstan, 2349 seeds were collected from 253 parent trees, a mean of 9.3 seeds per parent tree (Table 2.2, p.26). On average, 23 parent trees were sampled for each provenance collection, although six of the 11 provenances included 25 or more. The poor transport infrastructure of the region resulted in the collection of fewer provenances than planned but this was partially compensated by the collection of a higher average number of seeds per tree than was anticipated.

As the range of parent tree heights (Table 2.2) indicate, a wide range of age classes were sampled with a number of trees being less than 10 m, whilst some were over 30 m tall. Measurements of the height to first branch indicate a grand mean of 5.8 m for all provenances whilst only three provenances had individual means of less than 5 m of branch-free stem. The two provenances from Sary-Chalek, K10 and K11, had particularly high means for branch-free stem heights, both being over 7 m, whilst the maximum was over 14 m in K11 and nearly 18 m in K10 (tree 16) (frontispiece).

The mean for stem diameter at breast height across all provenances was 44.2 cm whilst the lowest provenance mean and smallest variation (Table 2.2) was for K1; where this reflects the relatively young age and homogeneity of the plantation. The provenances with the highest *dbh* means, K4 and K5, both contained many ancient trees estimated at over 200 years old (Popov, pers. comm.).

Branch angle data (Table 2.2) indicates that every provenance had a mean branch angle of '1' or less, *i.e.* 45^{o} or more from the stem. This result indicates the desirable phenotypic quality of the sampled trees, particularly in K11 where 63 % of the trees had branch angles greater than 60^{o} (scored as '0') from the stem.

Crown diameter measurements were not taken at all provenance locations. K1 had the lowest mean crown diameters, which would be expected due to the relative young age of the stand. In total, 71 trees were measured in order to sample a sufficient representation of tree age classes on a range of heterogeneous sites. The data were used to correlate crown diameter with *dbh* as in many species there is a significant relationship between the two parameters (Section 3.3).

The mean stem straightness within all provenances was a rank of 16 (Table 2.2, p.26) which indicates an equivalent of two utilisable timber lengths per tree; one of 3 m and one of 2 m (Appendix II, Table II.1). The plantation stand, K1, showed the highest mean ranking as

Table 2.2 Parent tree descriptors for the provenances collected in Kyrgyzstan.

Provenance		K1	K2	K3	K4	K5	K6	K7	K8	K9	K10	K11	TOTAL/ MEAN
Number of parent trees sampled		15	17	26	27	20	26	17	25	26	27	27	253
Total number of seeds collected		75	126	223	252	164	248	203	260	259	269	270	2349
Tree height (m)	mean (SD)	17.1 (4.1)	20.0 (4.8)	17.6 (3.9)	21.0 (4.5)	20.4 (5.2)	18.7 (5.0)	22.0 (4.9)	19.0 (4.1)	18.2 (5.0)	20.6 (5.6)	19.0 (6.6)	19.4 (1.5)
	range	9.3-24.0	14.5-30.4	12.9-31.0	13.0-28.1	10.8-31.3	6.7-28.4	12.9-30.3	10.2-26.7	9.1-29.9	11.3-34.0	10.1-34.0	6.7-34.0
Height to first branch (m)	mean (SD)	5.3 (1.3)	4.9 (1.7)	4.9 (1.7)	5.5 (2.8)	5.2 (2.8)	6.3 (3.0)	5.9 (2.3)	5.4 (2.4)	4.7 (1.7)	7.8 (2.9)	7.3 (2.5)	5.8 (1.0)
	range	3.5-7.1	2.2-9.3	2.2-8.6	1.5-12.0	1.9-12.4	1.8-16.4	1.9-9.0	1.0-10.8	2.5-9.7	3.8-17.7	4.0-14.4	1.0-17.7
dbh (cm)	mean (SD)	30.4 (7.7)	38.9 (13.0)	32.0 (7.0)	54.9 (27.8)	60.8 (30.8)	34.9 (11.3)	50.2 (18.5)	43.1 (19.9)	49.5 (21.3)	45.5 (29.6)	43.8 (30.5)	44.2 (9.5)
	range	20.4-45.2	24.0-61.5	19.1-46.1	17.1-102.0	19.9-116.0	12.8-67.2	24.7-91.9	18.7-107.3	16.7-87.4	17.0-128.3	14.0-112.7	12.8-128.3
Branch angle score [1]	mean (SD)	1.00 (0.53)	0.59 (0.51)	0.85 (0.46)	0.66 (0.50)	0.95 (0.22)	0.85 (0.67)	0.94 (0.66)	0.96 (0.54)	0.88 (0.52)	0.70 (0.54)	0.37 (0.49)	0.78 (0.19)
	range	0-2	0-1	0-2	0-2	0-1	0-2	0-2	0-2	0-2	0-2	0-1	0-2
Crown diameter (m)	mean (SD)	8.4 (1.8)	not	9.7 (1.9)	11.5 (3.7)	not	not	15.1	not	11.2 (3.0)	12.7 (3.1)	14.3 (0.8)	10.5 (2.4)
	range	5.6-11.2	measured	6.5-13.9	5.3-19.2	measured	measured	15.1	measured	6.2-16.9	7.5-15.2	13.7-14.8	5.3-19.2
Stem straightness rank [2]	mean (SD)	19.5 (5.8)	15.1(7.9)	13.9 (7.3)	16.1 (7.7)	16.2 (8.0)	17.6 (8.1)	14.1 (8.3)	14.8 (7.2)	13.1 (7.8)	18.7 (5.7)	19.7 (6.1)	16.3 (2.3)
	range	9-26	1-25	1-26	1-26	1-26	1-26	1-26	1-24	1-26	6-26	4-26	1-26
Burrs	% presence	0	5.9	0	40.7	40.0	11.5	17.6	24.0	46.2	22.2	22.2	22.1
Coppice	% presence	0	5.9	19.2	29.6	45.0	19.2	35.3	8.0	57.7	11.1	33.3	24.9

Branch angle score [1] measured from the stem : 0 = 90-60°, 1 = 60-45°, 2 ≤ 45°. Stem straightness [2] scored according to Barnes and Gibson (1986) (Figure 2.3).

would be expected although the degree of management of the stand was not known. Sary-Chalek provenances K10 and K11 were highly ranked with means of approximately 19. The exceptional tree in provenance K10 (frontispiece) not only scored the maximum of 26 for six straight metres but also was straight with a persistent leader beyond the first branch at 17.7 m.

The presence or absence of burrs varied considerably between provenances and indeed, burr presence is greater in those provenances with higher mean *dbh* measurements. Burr size and position varied considerably between trees; many trees had large root burrs (Plate 2.2). There were several indications in some provenances of burr removal by cutting, particularly in K4 and K5; these were said to have been the result of a British excursion to the area in the 1920s for the export of burr wood (Popov, pers. comm.). The trees were apparently left standing as the burrs were sliced off the side of the lower stem, leaving scars still visible today. The presence of coppice also varied between provenances but there was no correlation between the presence of burrs and the frequency of coppiced trees. Natural sprouting from old stems or damaged branches was evident in many provenances and it is hypothesised that this may be the main method of regeneration in the face of heavy overgrazing on the forest floor.

The analysis of leaflet morphology of each parent tree revealed that there was no variation between any of the 253 parent trees as all were classified as 'broad elliptic' (IPGRI 1994). Seed measurements and classification revealed some variation in seed mean sizes between provenances but more significantly indicated a greater degree of variation in seed shape (Table 2.3). The most common shape was 'round' (1), followed by 'long trapezoid' (6) whilst the other three shapes recorded were infrequent (Table 2.3).

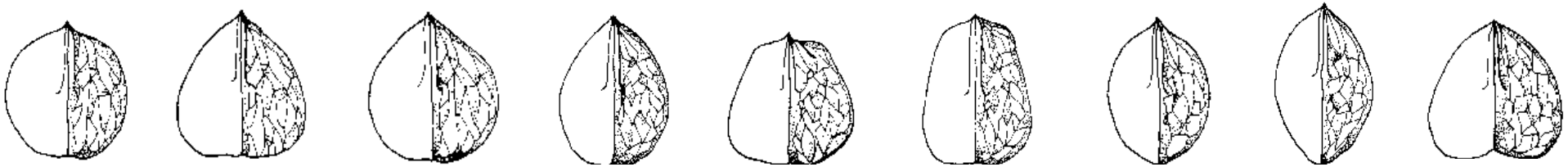
2.2.5 Discussion

The walnut-fruit forests of Kyrgyzstan are extensive and are present in diverse environmental conditions. They are variable in structure and contain a large variation in the form of individual phenotypes, as would be expected in natural forests. *Juglans regia* is not generally regarded a forest tree in Britain. Recently, published articles describing the walnut forests of Kyrgyzstan (Hemery 1998, Anon. 1999), including images of rolling hills and valleys clad in pure walnut forest, have evoked a strong response from British foresters. Contrary to established belief, *J. regia* does grow over a wide variety of site conditions and is able to form pure stands, to the exclusion of all other canopy species. Additionally, the species is able to grow impressively with a straight stem and fine branching habit, without any silvicultural intervention.

Table 2.3 Seed descriptors for the provenances collected in Kyrgyzstan.

Provenance		K1	K2	K3	K4	K5	K6	K7	K8	K9	K10	K11	Overall
Number of seeds collected		75	126	223	252	164	248	203	260	259	269	270	2349
Seed width (mm)	mean (SD)	29.0 (2.1)	28.6 (2.4)	26.0 (2.2)	28.5 (2.1)	28.6 (2.2)	27.1 (2.3)	28.4(2.6)	26.6 (2.3)	29.7 (2.0)	28.6 (3.8)	28.7 (2.1)	28.2 (1.1)
	range	24.6-32.2	22.7-34.4	20.7-29.5	25.0-33.9	25.0-32.7	23.4-34.6	23.8-32.8	22.3-32.0	26.1-33.8	22.4-38.6	24.4-33.2	20.7-38.6
Seed length (mm)	mean (SD)	35.1 (2.9)	33.3 (3.3)	30.8 (2.3)	31.9 (2.7)	32.5 (2.7)	31.7 (3.2)	33.0 (4.4)	29.4 (2.9)	33.2 (3.1)	31.4 (2.6)	32.2 (2.3)	32.2 (1.5)
	range	29.8-39.7	28.2-41.1	24.8-35.4	25.2-37.5	29.1-39.1	27.3-39.6	28.1-47.6	24.9-35.9	26.6-38.1	25.1-35.8	27.7-36.7	24.8-47.6
Seed shape [1] (%)	1	60	71	92	78	95	88	64	96	73	78	71	
	3	-	-	-	-	5	-	18	-	-	-	-	
	4	7	-	-	-	-	-	-	-	-	-	7	
	6	33	29	8	22	-	12	-	4	23	15	7	
	7	-	-	-	-	-	-	18	-	6	7	15	
Individual seed weight [2] (g)	mean (SD)	8.14 (1.35)	8.67 (1.97)	7.50 (1.32)	8.47 (1.65)	8.28 (1.28)	7.48 (1.27)	8.61 (1.84)	6.94 (1.72)	8.99 (1.08)	7.78 (1.22)	8.01 (1.36)	8.08 (0.61)
Mean number of seeds per kg [2]		123	115	133	118	121	134	116	144	111	129	125	124

Seed shape [1] IPGRI (1994)



1 Round **2** Triangular **3** Broad ovate **4** Ovate **5** Short trapezoid **6** Long trapezoid **7** Broad elliptic **8** Elliptic **9** Cordate

Seed weights [2] were measured after over-winter cold storage in the spring of 1998

A comprehensive database exists from the expedition (Appendix II, Table II.2) which is likely to be valuable in the longer term, when the phenotypic characters of the trees growing in the field trials (Chapter 5) may be correlated with provenance and parent tree details (Section 5.6). Within the timescale of this research project some morphological features of the young progeny, such as leaf flushing, survival and height increment, have been correlated with provenance site characteristics. Variables in seed morphology were also correlated with performance, particularly with seed germination and early transplant survival rates (Section 3.1).

The results presented here illustrate the inherent timber value of the walnut forests of Kyrgyzstan in terms of stem straightness, the quantity of naturally occurring branch-free stems and horizontal branching. A walnut stem with as little as 2 m of straight stem is of value in the present European timber market (Carizi and Molteni, pers. comm.). Each Kyrgyz provenance mean indicated at least 3 m of straight stem per tree. Some of the exceptional phenotypes, such as Tree 16 in K10, were not isolated specimens as many more excellent phenotypes were seen in the forest but if they were without seeds they were not recorded. Unfortunately, 1997 was not a good year for seed production in the Kyrgyz forests and often there was great difficulty in finding enough seeds.

The frequency of burrs in some provenance locations, often over 40 %, also indicates a huge market potential value in these natural forests. Unfortunately this potential has not been overlooked as, although the forests are protected areas, one American logging company had until recently been devastating a large area of allegedly ‘protected’ natural forest due to a mistaken agreement within the Kyrgyz bureaucratic system (Venglovsky, pers. comm.).

There are consequently strong arguments to consider the intraspecific variation within these forests so that estimates of genetic diversity may be used to address conservation at the molecular level. These data could then be used in conjunction with the more traditional approaches currently being undertaken in Kyrgyzstan, in terms of planning and structuring the management and protection of the forests (Carter 1997, Blaser *et al.* 1998).

2.3 Other genotype collections

Requests for material from foreign research organisations were normally met with generous assistance but ironically, some of the European countries closest to Britain proved to be the most difficult from which to obtain samples (*e.g.* Germany and The Netherlands). Attempts

to gain samples from Nepal, China and Uzbekistan were also unsuccessful. Some seeds were received from Hungary, Italy and the former Yugoslavia but in all these cases the quality of the seeds was poor resulting in low germination. Britain also proved to be difficult to sample. Letters were published in the British forestry press requesting information of the whereabouts of good walnut phenotypes but later in the year, many of the identified trees failed to produce seed.

Table 2.4 Summary of walnut collections.

Material code	Material name	Country of origin	Collection type	Parent tree identity	Material	Collector/Supplier	Organisation [1]
K1	Ak-Terek	Kyrgyzstan	Provenance	✓	Seed	Gabriel Hemery	Researcher
K2	Ak-Terek	Kyrgyzstan	Provenance	✓	Seed	Gabriel Hemery	Researcher
K3	Ak-Terek	Kyrgyzstan	Provenance	✓	Seed	Gabriel Hemery	Researcher
K4	Sharap	Kyrgyzstan	Provenance	✓	Seed	Gabriel Hemery	Researcher
K5	Yaradar	Kyrgyzstan	Provenance	✓	Seed	Gabriel Hemery	Researcher
K6	Shaidan	Kyrgyzstan	Provenance	✓	Seed	Gabriel Hemery	Researcher
K7	Kyzyl-Ungur	Kyrgyzstan	Provenance	✓	Seed	Gabriel Hemery	Researcher
K8	Katar-Yangak	Kyrgyzstan	Provenance	✓	Seed	Gabriel Hemery	Researcher
K9	Kyok-Sarau	Kyrgyzstan	Provenance	✓	Seed	Gabriel Hemery	Researcher
K10	Kyr-Sai	Kyrgyzstan	Provenance	✓	Seed	Gabriel Hemery	Researcher
K11	Ters-Kolt	Kyrgyzstan	Provenance	✓	Seed	Gabriel Hemery	Researcher
E1	Catalonia	Spain	Landrace	✓	Seed	Neus Aletà Soler	IRTA
J1	Tajikistan	Tajikistan	Seed source*	✓	Seed	Eric Germain	INRA
P1	Karaj	Iran	Seed source*	✓	Seed	Eric Germain	INRA
R1	Romania	Romania	Landrace	✓	Seed	N.-Valeriu Nicholescu	UT
S1	Slovakia	Slovakia	Landrace	✓	Seed	Dusan Sojak	LVU
T1	Trabzon	Turkey	Provenance	✓	Seed	Hasret Atasoy	DKOAM
T2	Anatolia	Turkey	Provenance	✓	Seed	Sitki Ugurlu	BAORAM
B1	Rossosh	Russia	Seed source	✗	Seed	Alexander Sychov	RZHES
B2	Kourpat	Ukraine	Seed source	✗	Seed	Alexander Sychov	RZHES
A1	Cauc 26	Georgia	Seed source*	✗	Transplant	Bruno Fady	INRA
F1	RA464 Lozerrone	France	Landrace	✗	Seed	Eric Germain	INRA
F2	RA611	France	Landrace	✗	Seed	Eric Germain	INRA
F3	Charrier	France	Landrace	✗	Transplant	Jacques Becquey	IDF
U1	UK	United Kingdom	Landrace	✓	Seed	Gabriel Hemery	Researcher

ORGANISATIONS [1]

BAORAM	Bati Akdeniz Ormancilik Arastirma Müdürlügü	*Southeast Anatolia Forest Research Institute, Turkey*
DKOAM	Dogu Karadeniz Ormancilik Arastirma Müdürlügü	*Eastern Black Sea Forest Research Institute, Turkey*
IDF	Institut pour le Développement Forestier	*Institute for the Development of Forestry, France*
INRA	Institut National de la Recherche Agronomique	*National Institute for Agronomic Research, France*
IRTA	Institut de Recerca I Tecnologia Agroalimentaries	*Institute for Agronomic Research and Technology, Spain*
LVU	Lesnicky Vyskumny Ustav	*Forest Research Institute, Slovakia*
RZHES	Rossosh Zonal Horticultural Experimental Station	*Russia*
UT	University of Transylvania	*Romania*

Seed source* denotes a collection amassed from a seed orchard.

By the close of 1997, 25 sources of material had been amassed, including the 11 provenances from Kyrgyzstan (Table 2.4). The material from Tajikistan (J1), Iran (P1) and the Caucasus (A1) was obtained from existing collections of the French organisation INRA. The seed was

produced from open-pollinated seed orchards planted in France from which cross fertilisation with other walnut origins had been minimised (Germain[5], pers. comm.). The number of original trees from which the collections were made is unknown. The collections from Russia (B1) and the Ukraine (B2) were bulked seed samples from local trees. The French collections represented named material that is currently widely used in the country: Lozerrone RA464 (F1) is a late-flushing and vigorous genotype and is often used as a rootstock (Fady[6], pers. comm.), RA611 (F2) is a vigorous selected progeny (Germain, pers. comm.) and 'Charentes' (F3) is a population selected for late flushing and vigour (Becquey[7], pers. comm.).

The most thoroughly gathered material supplied by foreign collectors, involving 20 or more parent trees and the maintenance of progeny identity, was from Romania (Valeriu Nicolescu), Slovakia (Dusan Sojak), and the two provenances from Turkey (Hasret Atasoy and Sitki Ugurlu) (Table 2.5). Seed material from some other countries (E1, J1 and P1) was more difficult to gather resulting in a low number of parent trees (Table 2.5). All material from other sources was bulked (*i.e.* no information on the parent trees was available) and in two cases material was supplied as two year-old transplants rather than seed (Table 2.4).

The appropriate classification for the material is country-dependent because each collection was undertaken differently. The term 'provenance' as used for the Kyrgyzstan provenances may only be extended to the Turkish provenances, although strictly these were not composed of single communities of interbreeding trees as these populations were not contiguous. For simplicity, the looser definition for 'provenance' from Helms (1998) may be used: *the original geographic source of seed*. Material collected from the other countries must be defined by alternative means and these are summarised in Table 2.4. The term 'land race' is defined by Helms (1998) as: *a population of plants, usually exotic, that has become adapted to a specific environment.* The term 'seed source' is used here to cover all other origins as this refers to the locality from where the seed was collected (Helms 1998) and makes no reference to true origin.

The information accompanying each seed collection was variable in quality. Romanian and Slovakian seed collections were supplied with details of the parent trees' locations by region and altitude (Appendix II, Tables II.3 and II.4). The information supplied for Turkish provenance T1 was the most thorough and consisted of details of the five locations within the

[5] Eric Germain, Deputy Director, UREFV.

[6] Bruno Fady, Director, Institut National de la Recherche Agronomique, France.

[7] Jacques Becquey, Institut pour le Développement Forestier, France.

Trabzon region where trees were sampled and details of each parent tree, namely total height, *dbh* and estimated age (Appendix II, Table II.5). Seeds from the Turkish provenance T2 were collected from 25 trees spaced 50 m apart within a stand of trees for which general environmental variables were supplied (Table 2.5).

Table 2.5 The 25 provenance locations and number of parent trees sampled.

Material code	**Material name**	**Country of origin**	**Latitude** (°N)	**Longitude** (°E)	**Altitude** [1] (m)	**Number of trees sampled**
K1	Ak-Terek	Kyrgyzstan	41.25	72.75	1700	11
K2	Ak-Terek	Kyrgyzstan	41.25	72.75	1390	17
K3	Ak-Terek	Kyrgyzstan	41.25	72.75	1860	26
K4	Sharap	Kyrgyzstan	41.25	72.75	1620	26
K5	Yaradar	Kyrgyzstan	41.25	73.00	1260	20
K6	Shaidan	Kyrgyzstan	41.25	72.75	1590	26
K7	Kyzyl-Ungur	Kyrgyzstan	41.40	73.00	1400	17
K8	Katar-Yangak	Kyrgyzstan	41.25	72.75	1900	24
K9	Kyok-Sarau	Kyrgyzstan	41.25	72.75	1830	26
K10	Kyr-Sai	Kyrgyzstan	41.75	72.00	1320	27
K11	Ters-Kolt	Kyrgyzstan	41.75	72.00	1440	27
E1	Catalonia	Spain	42.00	3.00	175	5
J1	Tajikistan	Tajikistan	38.95	70.00	*	7
P1	Karaj	Iran	36.28	52.00	*	5
R1	Romania	Romania	45.84	25.90	478	27
S1	Slovakia	Slovakia	48.24	18.96	215	34
T1	Trabzon	Turkey	39.98	40.00	776	25
T2	Anatolia	Turkey	38.70	42.45	1650	21
B1	Rossosh	Russia	50.25	39.50	95	bulked
B2	Kourpat	Ukraine	48.00	25.50	350	bulked
A1	Cauc 26	Georgia	43.00	45.00	*	bulked
F1	RA464 Lozerrone	France	48.00	0.00	*	bulked
F2	RA611	France	48.00	0.00	*	bulked
F3	Charrier	France	48.00	0.00	*	bulked
U1	UK	United Kingdom	53.00	0.00	*	4
					SUM	**375**

Altitude [1] an asterisk denotes that data were not made available.

2.4 Discussion and conclusions

The extensive natural walnut-fruit forests in Kyrgyzstan are likely to provide an important genetic resource for a species that has a fragmented distribution throughout most of its current range and has been strongly influenced by anthropogenic activities. A current lack of knowledge regarding the genetic diversity of *Juglans regia*, in combination with the

hypotheses of the loss of genetic diversity across some parts of its range has pointed to the importance of implementing a range-wide study of genetic variation.

The collected populations provide a representative sample of material across the natural and introduced ranges of *Juglans regia.* Analyses of this material using molecular markers (Chapter 4) should provide an estimate of intraspecific genetic variation and indicate how this diversity is distributed. If genetic diversity is low in the current range this may indicate the importance of the conservation of *J. regia* in its natural range in order to conserve the residual genetic variation of the species. Studies of intraspecific variation in chestnut (*Castanea sativa*) (a species similar to walnut in that it has been widely cultivated, particularly by cloning and grafting) indicate that the species has suffered a decline of genetic variation as a result of anthropogenic influence (Fineschi and Malvolti 1991). One of the consequences for chestnut has been the devastating spread of pathogens, particularly *Endothia parasitica* (Murrill) M. Barr (chestnut blight). In some plantations the species has been completely eliminated except for some regeneration from the rootstocks which, containing a greater degree of natural genetic variation than the clonal tree grafts, have survived as coppice (Fineschi and Malvolti 1991). The indigenous forests of a species are a source of genetic diversity for adaptation to new environments, a source of resistance to diseases and pests, and a source of variation to adapt to new silvicultural practices or timber products (Frankel *et al.* 1995).

If the study of genetic diversity within this project (Chapter 4) indicates that *Juglans regia* has the high intraspecific genetic variation that is exhibited in most tree species (Hamrick *et al.* 1992), this will indicate the importance of capturing and maintaining this diversity by conservation and management strategies. An understanding of the structure and distribution of genetic variation is therefore of critical importance. The loss of genetically distinct populations within a species is as important and irreversible as species extinction itself, and therefore a fundamental compromise on the future options of both present and future generations. It is hoped that within this project, the value of the walnut forests of Kyrgyzstan and of other the sampled populations, in respect to their contributions to the global walnut gene pool, may be estimated for the first time. This knowledge may be applied as a new parameter in the selection of areas of existing walnut forest to conserve through the development of *in situ* conservation programmes. The collected genotypes will not only provide the opportunity to improve the species' potential as a timber-producing tree in the UK but also play an important role in identifying and enhancing the conservation of the species on a global scale.

Chapter 3 Silviculture

3.1 Nursery techniques

3.1.1 Introduction

The walnut seeds gathered for the studies on genetic variation and provenance performance, as described in Chapter 2, were propagated at a commercial tree nursery[1] in north Oxfordshire using some innovative nursery techniques. A brief literature review of walnut nursery practice is included below, followed by a description of the techniques adopted for this project. The results of the nursery stage are not fully supported statistically but do provide some useful indicators for further research.

Walnut seed generally has a high viability (Table 3.1) (Aldhous 1972), although small nuts often fail to germinate, so if they predominate in a seed lot, low germination yields must be expected.

Table 3.1 *Juglans regia* seed viability.

No. cleaned seeds/kg		Average	Average	Average	Average no.	Source*
range	average	purity %	viability %	germination %	viable seed/kg	
30-197	100	100	84	80	84	1
65-180	--	100	-	75	-	2
78-233	129	100	--	72	93	Kyrgyz seed

* Sources: 1 Gordon and Rowe (1982); 2 Aldous (1972); Savill (1991)

Seeds of *Juglans regia* have been successfully stored at 20 to 40 % moisture content (m.c.) in plastic bags at 4 ^{o}C for one year whilst seeds with 50 % m.c., placed in screen containers in an outdoor pit, have survived for four years without significant loss to germination capacity (Young and Young 1992).

Most species of the *Juglandaceae* have a dormant embryo and, to break dormancy, they require a period of cold storage followed by stratification. The stratification time recommended for *Juglans regia* is in the range of one to five months (Dirr and Heuser 1987). Gordon and Rowe (1982) recommend that seed at 40 to 45 % m.c. should be mixed with damp peat and stored at 0 °C in a location that retains moisture but allows ventilation. Fungicides may be applied to the seed to prevent diseases during cold storage. Germination can be induced by a rise of temperature to 20 °C for 16 hours followed by 30 °C with light, for eight hours (Gordon and Rowe 1982).

[1] Nicholson Nurseries, Steeple Aston, Oxfordshire.

Sowing may be done in open ground or in containers, in which case 'root-trainers' are particularly recommended, with the seed being transferred to the pots shortly after germination (Crawford 1996). The seeds should be covered by 25-50 mm of moist sandy compost (Gordon and Rowe 1982).

In most nurseries, walnuts grown and sold for planting in woodlands are usually 2+2 transplants although 1+1s are also acceptable. For nut production, clones and grafted trees are commonly recommended. Successful nursery practice requires a soil with a near-neutral pH that has been well fertilised prior to sowing. Root pruning is common in the nursery due to the length of the tap root, which may grow down to one metre in the first year (Popov, pers. comm.) although there is a great deal of contrasting advice. Popov (1981) reported that systematic pruning or accidental injury to the tap root will result in an increased susceptibility to disease. Advice for British nurseries continues to be the production of 2+0 plants, although particular reference is made to caring for the roots, which should be carefully trimmed if torn during undercutting (Aldhous 1972). In the nursery the transplants should be planted at 60 × 60 cm spacing and those with a diameter above the root collar greater than 20 mm, should be stumped back to 25mm above the root collar to ensure vigorous leader growth (Gray 1939, Aldhous 1972). The transplants should be left in lines for a further two years during which any lateral growth should be pruned in July/August (Aldhous 1972).

3.1.2 Methods

The methods adopted for the propagation of the walnut seeds in this project (spring 1998) differed in a number of ways from some of the recommended protocols summarised above. Some unpublished techniques were adopted and although not rigorously tested under experimental conditions, were applied due to the constraints of time and certain research needs.

The seeds were collected as described in previous chapters, transported and stored in breathable bags in a cool and dry environment (between 0 °C and 12 °C), as recommended by Popov (pers. comm.). The mean seed weight within each seed lot (provenance/progeny) was calculated with a view to assessing seed viability in relation to seed weight.

The large number of seeds (*c.* 3000) and the problems associated with maintaining the identity of approximately 400 small seed lots, called for a simpler approach to inducing germination than that possible by stratifying the seeds. Gibberellins, which are able to break dormancy and induce uniform germination in some seeds (Lawrence 1995), have been successfully used with walnuts in

Spain since 1995 (Aleta-Soler[2], pers. comm.). The following protocol was adopted, as supplied by Aleta-Soler (pers. comm.): each seed was partially opened along its basal joint with a blunt knife and soaked in GA_3 gibberellic acid (Sigma G-7645) at 100 ppm for 48 hours. The gibberellic acid is absorbed by the seeds during soaking by imbibition. Thereafter the gibberellins stimulate growth by inducing the synthesis of enzymes that convert starch into sugars (Lawrence 1995). The individual seed lots were labelled and packaged in net bags for soaking, which was carried out in two standard dustbins (approximate volume of 100 litres). The seeds were drained of all excess fluid before sowing directly into the nursery bed.

The difficulties associated with substantial tap root growth in the nursery (Section 3.1.1), including root pruning which has been shown to be detrimental to tree health (Popov 1981), highlighted the need to adopt a novel approach to nursery bed design. Pot sowing was ruled-out as there were no suitable pots with an adequate depth (35 cm) available on the market within budgetary constraints. The usual disadvantage of open sowing in a nursery bed is the difficulty of lifting due to root depth. Walnuts have a reputation of being difficult to transplant successfully from the nursery to their final position because of the length of the tap root and the slow development of the fibrous root system (Popov 1981, Savill 1991). In France, walnut seedlings are grown in open beds where they are undercut at depths of over 60 cm, and sold as 1u1s (Becquey, pers. comm.). After consideration of these factors, a technique was developed, adapted from a method used originally in Italy (Ducci, pers. comm.).

The bed was designed to produce seedlings with a maximum root length of 40 cm that would be easy to lift without a requirement for undercutting. A raised bed with a soil depth of 35 cm was created, overlying a 5 cm layer of wood chips spread over a 'Mypex' membrane (Figure 3.1). The 'Mypex' membrane is a permeable fabric that excludes weed growth from below ground, and may form an additional barrier to tap root growth. The soil constituents were a combination of peat and sand in a 3:1 ratio. The peat was both moss and sedge types mixed to achieve a pH of 7.0. The peat/sand combination was selected because of its friable nature, which would provide free-draining conditions and ease lifting. The soil included the proprietary fertiliser 'Osmocote Plus'[3] in a dual mix of quick and slow release forms (5-6 months and 12-14 months respectively), both of which were included at a rate of 2 kg m^{-3} soil. Coarse wood chips ranging from 30 to 50 mm in size, were included as a natural air-pruning medium to limit the downward growth of the roots to between 35 and 40 cm. This depth was deemed a practical limit for easing the effort

[2] Neus Aleta-Soler, Plant Breeder, Institut de Recerca i Tecnologia Agroalimentàries, Spain.

[3] 'Osmocote Plus' constituents were N, P, K, and Mg: 5-6 month form = 15, 10, 12, 2 (mg/l) respectively
12-14 month form = 15, 9, 11, 2 (mg/l) respectively.

required when lifting the seedlings for planting in their final positions.

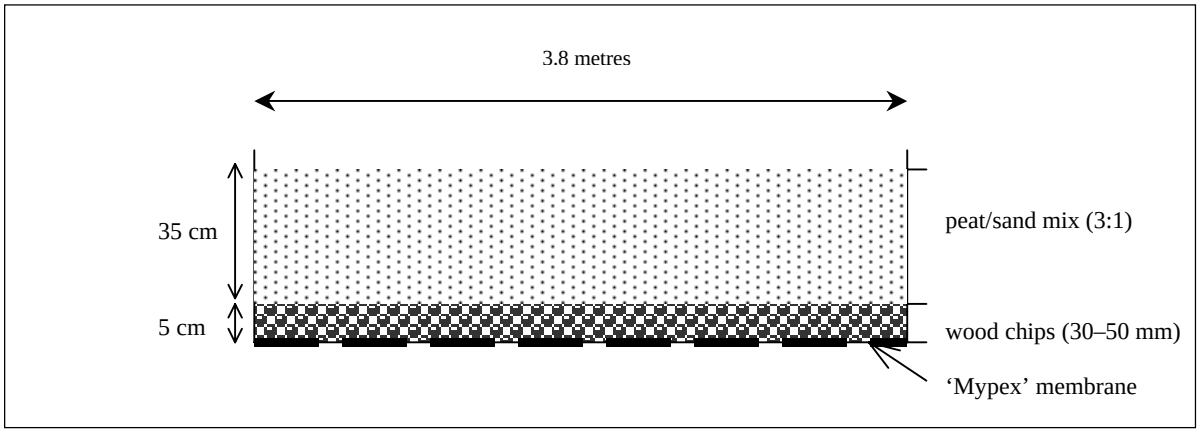


Figure 3.1 Schematic diagram of nursery bed construction.

The prepared bed was protected by a tunnel of clear polythene on curved supports with mesh windbreaks at either end. The use of the polytunnel allowed the seeds to be sown before the danger of frost had passed, thereby extending the growing season of the seedlings beyond that possible if they had been grown in the open. The seeds were sown in mid March at a depth of twice the width of the individual seed, typically 5 to 7 cm deep, at a spacing of 10 × 20 cm. The bed measured approximately 20 × 4 m and was divided into two halves. Each provenance, but not progeny, was divided equally and distributed randomly in each half of the bed. This not only provided opportunities for a statistical analysis of seed viability and seedling survival, but also created a buffer from accidental damage that would otherwise have had the potential to devastate whole provenances.

The daily amount of water supplied to the bed, by an overhead and pressurised sprinkler system, was measured. The decision of when to water was made by an experienced nursery practitioner, based on a subjective assessment of soil moisture at 3 to 5 cm below the soil surface. Between the time of sowing until the removal of the polytunnel cover, a total of 367 mm of water was applied, averaging a little over 4 mm per day. Minimum and maximum temperatures inside the polytunnel were recorded during the same period. The minimum temperatures ranged from –2.0 to + 14.0 °C, averaging 5.9 °C, whilst maximum temperatures ranged from 10.0 to 40.0 °C, averaging 27.8 °C. Once the danger of frost was judged to have passed in early June, the polythene cover was initially vented by cutting several 50 cm diameter holes along each side. Apart from a one metre high wall of polythene that was retained to provide some shelter from winds, the cover was completely removed in mid-June. Irrigation was halted in early September

to allow the seedlings to harden before leaf fall and the onset of autumn frosts, as recommended by Ducci (pers. comm.).

The seedlings were lifted from the nursery bed in late September once leaf-fall was complete. The stem heights and diameters (at the root collar) of the seedlings were measured to the nearest 1 mm. Data on seed germination and seedling growth were analysed using Minitab[4].

3.1.3 Results

The first seedlings were visible above ground approximately 50 days after sowing. By day 61, 41 % of the seeds had germinated, rising to 57 % by day 69 and 68 % by day 83. The final assessment, made at the end of the growing season, indicated a germination percentage of 72 % which is slightly lower than those presented in Table 3.1, of between 75 and 80 %.

Table 3.2 Summary statistics of walnut tree heights and stem diameters after the first growing season in the nursery.

Provenance		No. seeds	Seedling height (cm)				Stem diameter (mm)			
No.	Code		Mean	StDev	Min	Max	Mean	StDev	Min	Max
1	K1	23	8.8	3.3	2.5	14.0	8.6	4.9	1.0	21.0
2	K2	95	9.7	3.4	2.5	21.0	9.0	3.8	2.0	20.0
3	K3	152	10.4	2.8	4.0	19.0	9.5	3.7	4.0	25.0
4	K4	161	10.7	5.3	2.5	44.0	10.1	4.3	2.0	31.0
5	K5	140	11.1	4.1	4.0	29.0	9.4	3.7	1.0	22.0
6	K6	184	12.0	5.5	3.0	52.5	9.9	3.9	3.0	22.0
7	K7	151	10.7	5.1	2.5	44.0	9.2	4.1	2.0	23.0
8	K8	167	9.6	3.1	1.0	23.0	8.5	3.4	2.0	21.0
9	K9	190	11.6	4.2	2.0	33.0	9.8	3.9	2.0	24.0
10	K10	208	10.7	4.3	3.5	50.0	9.2	3.8	2.0	26.0
11	K11	191	10.1	3.3	2.5	20.0	9.0	3.6	2.0	23.0
12	E1	44	16.5	7.2	5.0	39.0	13.4	5.2	5.0	33.0
13	J1	60	13.0	5.7	3.0	31.5	14.4	4.8	3.0	25.0
14	P1	38	14.2	5.6	6.0	27.0	13.0	4.2	6.0	23.0
15	R1	63	13.0	5.8	4.0	40.0	10.9	4.8	4.0	24.0
16	S1	107	13.8	7.7	3.0	41.0	10.6	4.6	1.0	23.0
17	T1	50	20.4	19.4	4.5	86.5	12.4	5.9	2.0	27.0
18	T2	38	13.1	7.4	3.5	31.5	11.9	6.0	3.0	25.0
19	B1	10	10.4	3.3	6.0	18.5	7.6	2.5	4.0	13.0
20	B2	8	12.8	4.8	6.0	19.0	9.3	4.3	5.0	17.0
22	F1	24	16.7	10.1	8.0	56.5	10.9	3.4	5.0	18.0
23	F3	15	15.2	7.4	8.0	35.0	11.0	2.6	5.0	14.0
25	U1	16	13.5	6.3	5.5	27.0	10.6	3.6	4.0	18.0
	Overall	2135	11.6	6.0	1.0	86.5	9.8	4.2	1.0	33.0

Specific analyses of seed germination were only undertaken for the Kyrgyz material, as these were the only seed lots for which the correct handling procedures from the time of collection to storage, could be guaranteed. Correlation between means of seed weight and final germination ratio for progeny seed lots was tested using Spearman's rank correlation coefficient but was non-

[4] Minitab Release 11, Minitab Inc., 3081 Enterprise Drive, State College, PA 16801-3008, USA.

significant. The mean weight for individual seeds (progeny seed lots) was 8.0 g and ranged from 4.3 to 12.8 g, which is lower than those reported in other studies (Table 3.1). Seedling survival through the growing season was impressively 100 % although a few of the seedlings (< 0.5 %) had surprisingly survived the entire growing season without producing roots, using nutrients stored within the seed. There was no significant correlation (Spearman's rank correlation coefficient) between mean seed weight and seedling height or stem diameter for the progenies.

Seedling size was measured for all of the provenances and was wide ranging in both height and stem diameter (Table 3.2). Differences between the provenances, for both parameters, were highly significant (p<0.001). A multiple comparison of means was undertaken with the Tukey test (Hoppe 1993). Within the Kyrgyz provenances (K1-K11) as a group there were no significant differences in either height or diameter means. Provenance T1 (Turkey 1) was significantly (p=0.001) taller than all but five (E1 (Spain), B2 (Ukraine), F1- F2 (France) and U1(UK)) of the other 24 provenances (Figure 3.2).

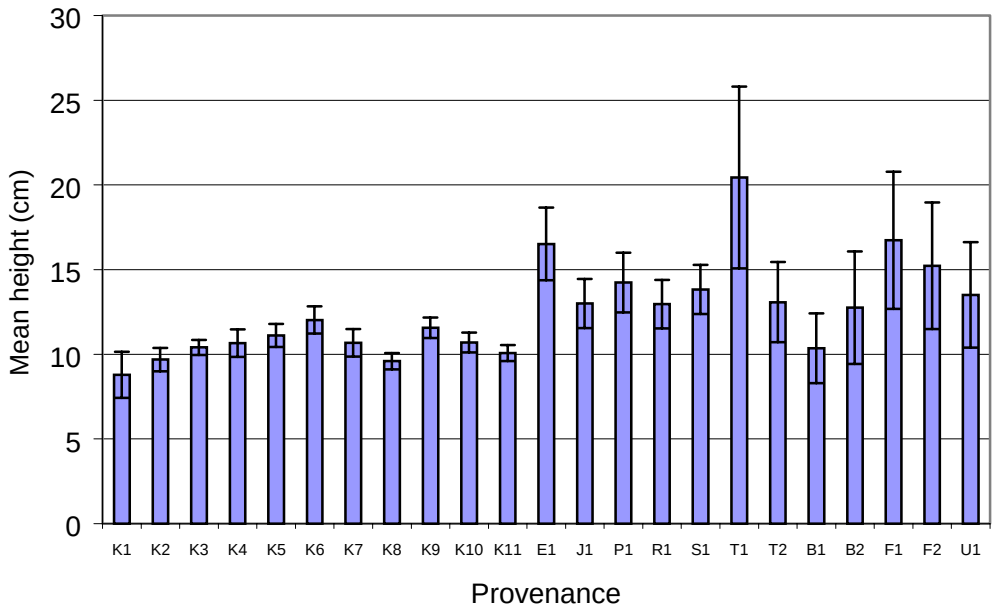


Figure 3.2 Mean heights of provenances after one year in the nursery. Error bars show 95 % confidence limits.

There were fewer significant differences for stem diameters between provenances. Within the Kyrgyz provenances as a group, there were none. Stem diameters for provenances E1, J1, P1, T1 and T2 were significantly (p<0.001) greater than for many of the Kyrgyz provenances, although differences within these five provenances, and between the other non-Kyrgyz provenances, were non significant.

3.1.4 Discussion and conclusions

The nursery stage of this project was not designed as an experiment and therefore no fully supported recommendations can be made. Nevertheless, a number of useful indicators for the simplification and improvement of walnut silviculture in the nursery have been identified and these could be pursued in future research.

The use of gibberellins in this instance did not cause any change from the germination percentage expected from the literature for stratified seeds. However, their use provided an extremely practicable means of handling a large number of small seed lots and of promoting germination within a limited time when the adoption of traditional and lengthy stratification methods would have been unacceptably limiting. Further studies could usefully be conducted to analyse the effectiveness of gibberellins in promoting walnut germination, especially by drawing comparisons with traditional methods. Their application on a commercial scale may be limited by the need partially to open every seed, unless this technique were deemed unnecessary, perhaps in conjunction with a longer soaking period. Alternatively, a suitable mechanical method could be used to open the seeds.

The poor correlation between seed weight and germination success reported here is likely to have been influenced by efficient pre-screening of the seed, involving the disposal of lightweight seed. Further analysis of the seedlings' first year growth in comparison with alternative nursery techniques is impossible, as data are unavailable for the latter. One notable observation from the nursery bed was that many of the tallest seedlings appeared to be growing in positions where their nearest neighbours had failed to germinate. This supports the view that the spacing at which the seed were sown (10 × 20 cm) was too close and that, where there was less root competition, seedling growth was superior. Indeed a spacing of 20 × 20 cm was initially desired but constraints on space, due to the large number of seeds, had necessitated the closer spacing.

An important success resulting from the nursery bed design was the root structure of the seedlings. A highly fibrous root system was promoted which, in the author's experience, was superior to root systems produced by traditional techniques. The air pruning medium successfully limited tap root growth to 35 cm, either by promoting vigorous fibrous root growth at this depth, or less often by diverting the tap root horizontally. This greatly eased the transplanting of the trees to their final positions, as a planting hole of only one spade depth was necessary. The friable nature of the nursery soil made the lifting of the seedlings easy, even though weather conditions at that time were wet.

3.2 Establishment techniques

3.2.1 Introduction

PLANTATION DESIGN

Walnut is usually planted as a monoculture in open groves, as recommended by many authors (*e.g.* MacDonald *et al*. 1957). Evans (1984) stated that it may also be grown in plantation conditions where the trees should be planted at wide spacing with 1100 to 625 trees per hectare (ha) (3 to 4 m spacing), aiming for a final crop density of 40 to 70 trees ha^{-1} (12 to 15 m spacing). Evans (1984) warned against planting walnuts with other species of the same age, due to their intolerance to over-shading from above and from the side, unless the accompanying nurse species is very carefully maintained.

There is however, increasing evidence that walnut may benefit when planted with suitable nurse or companion trees and shrubs. In the USA, Schlesinger and Williams (1984) tested black locust (*Robinia pseudoacacia* L.), autumn-olive (*Elaeagnus umbellata* Thunb.) and alder (*Alnus glutinosa* P.Gaertn) in mixtures with black walnut (*Juglans nigra*). These species were chosen for their nitrogen-fixing (*N*-fixing) capabilities because many hardwood trees, including *J. nigra*, have shown improved growth when grown with *N*-fixing species (Finn 1953). Schlesinger and Williams (1984) found that all of the nurse species increased walnut height growth but that the treatment effect was site dependent. Walnuts interplanted with *Elaeagnus* resulted in height gains of 56 % to 351 % over non-nursed walnuts. Problems with rapid growth of the *Robinia* necessitated severe control of its height by coppicing or ring-barking. There was high mortality of the alder nurse after 5 years, allegedly due to an allelopathic reaction with the walnut.

Friedrich and Dawson (1984) analysed soil nitrogen concentration under *N*-fixing crops with *Juglans nigra* in southern Illinois. Soil nitrogen concentration was highest under nurse crops of *Robinia pseudoacacia* and *Elaeagnus umbellata*, with decreased amounts under *Alnus glutinosa* and lower still under *Lespedeza striata* Thunb. Campbell and Dawson (1989) calculated projections of growth that showed average *dbh* values of 28 cm in 31 years for walnut inter-planted with the *Elaeagnus*. They projected that 40 years of growth would be required for the walnut to achieve 28 cm *dbh* with an alder nurse or 80 years for those with no nurse. *Elaeagnus* produces a multi-stemmed understorey reaching less than 6 m in height, with the benefit of reducing weed competition beneath its dense canopy.

In central Italy, mixed plantations with *Juglans nigra* established in 1985, have demonstrated similar impressive growth (Buresti and Frattegiani 1994). More recently, plantations with *J. regia* mixed with cherry (*Prunus avium* L.), *Elaeagnus angustifolia* P. Blanco, *Alnus cordata* Desf. and

Robinia pseudoacacia have been established (Buresti 1995). Six year results indicated that walnut increased in height by 48 % and stem diameter by 36 % when planted with cherry (non *N*-fixing) compared to pure walnut plantings, whilst with the other (*N*-fixing) nurses, increased by 76 % and 42 % respectively (Buresti 1995).

Campbell and Dawson (1989) also proposed that requirements for corrective pruning of walnut trees could be reduced with the use of a suitable nurse. It has been hypothesised that the use of nurses may help control walnut anthracnose *Mycosphaerella* spp. by inhibiting the bacterium's spore movement from infected walnut leaf litter (Kessler 1985). Li *et al.* (1967) proposed that *Alnus rubra* Bong. may have a potential in the biological control of root pathogens such as *Poria weirii* Murr. and the honey fungus (*Armillaria mellea* Vahl *ex* Fr.). *Alnus* spp. fix atmospheric nitrogen in nitrate rather than ammonium or amine forms, and these root pathogens cannot use nitrate nitrogen, unlike antagonist organisms such as *Streptomyces* spp. which thrive on nitrate (Li *et al.* 1967).

PLANTING

The size of the planting stock is an important consideration for the successful establishment of walnut. Evans (1984) recommends that a transplant should be 70-90 cm tall with a root collar diameter of 15-20mm. Traditionally 2+2 transplants are used in forestry (Aldhous 1972, Evans 1984). Transplants of 1+1 may be used with care, as they are more prone to frost damage and drying out (Evans 1984). Evans (1984) recommended that walnut should be planted in November although this could be delayed until mid-March, as is the case with most broadleaved species. Careful pit-planting is suggested.

Populations produced by grafting are commonly used in nut production but the use of grafted material for timber production is of dubious benefit and their use may result in a degradation of timber value (Natale *et al*. 1993). However, the majority of valuable burrs currently come from grafted trees (*Juglans regia* on *J. nigra* rootstocks) in North American nut orchards. The increased risk of disease associated with grafting would be more difficult for the forestry manager to control, who traditionally would not as readily resort to the use of fungicides as the horticulturist.

STUMPING AND PRUNING

Stumping is the practice of cutting back the stem to just above ground level at planting time, or within the early years of establishment, to stimulate a vigorous new shoot. It was once a common technique for establishing some species in Britain (Hibberd 1988) which may have been documented as 'coppicing' in some instances (Pope and Mayhead 1994). It can only be used on

those species that can regenerate from dormant buds. Stumping is undertaken for a variety of reasons:

- reducing the bulk of material thereby making transport easier;
- reducing post-planting stress and improving initial survival;
- improvement of the root to shoot ratio;
- promoting a straighter and more vigorous main leader;
- as a corrective treatment following damage by browsing, fire or frost.

In walnut silviculture, the most important benefit of stumping is in its promotion of rapid height increment through the early frost-sensitive phase of growth (Pope and Mayhead 1994). A stumping treatment for walnut establishment has been recommended for 2+0 plants (MacDonald *et al*. 1957, Aldhous 1972), while Gray (1939) and MacDonald *et al*. (1957) recommended a further two years in the nursery transplant bed (2+2). Edlin (1945) recommended stumping at one year. Between 1938 and 1942 five walnut experiments were established by the Forestry Commission at Ffosydd Orles in Wales using *Juglans regia* and *J. nigra.* The trials, which tested the effect of stumping, concluded that it was a 'very risky practice' which could not be recommended (Forestry Commission, unpublished). At these trials, mortality of stumped material after six years was 2.5 times greater than for unstumped; the main risk was believed to be the effect of repeated frosting before the young shoots could grow beyond the frost zone. Pope and Mayhead (1994) analysed the effect of stumping on two-year-old *J. regia* seedlings and transplants, and found that the treatments had no significant effects. Snellgrove and Mayhead (1995) studied the effect of stumping on different walnut plant types, concluding that a stumping treatment caused a vigorous response in terms of height increment, as stated by Evans (1984).

Walnut does not have a strong central axis or apical dominance as it has a sympodial growing habit (Evans 1984). Allaby (1992) defines sympodial as 'a type of branching in which an apparent main axis is made up of many lateral branches, each arising from the branch before'. Consequently, formative pruning is a prerequisite in walnut silviculture. Singling, the practice of reducing shoots to one selected as a leader, is likely to be an essential management activity following stumping (Snellgrove and Mayhead 1995). Evelyn (1678) proposed that pruning should be done in early September; (' *..for at that [time] the trees are not subject to bleed'*) and so that the wound may heal before temperatures fall. More recently, Evans (1984) and Becquey (1997) recommend pruning in the summer, because excessive sap weeping occurs when pruning takes place in the spring. These recommendations are similar to those for some other broadleaved species, particularly cherry (*Prunus avium*) (Kerr and Evans 1993b). Crawford (1996) concurs

with the recommendation for summer pruning but adds that walnuts can also be pruned whilst they are completely dormant.

As stated above the practice of root pruning is common, both in the nursery and at planting time, due to the difficulties of planting with the long taproot that may typically extend below spade depth. Popov (1981) reported that systematic pruning or injuring to the taproot of *Juglans regia* will cause an increased susceptibility to disease and rotting. He also reports that the taproot, once pruned, will not re-grow but instead will be replaced by fibrous roots that grow considerably slower.

TREESHELTERS

Treeshelters have been widely adopted in lowland forestry in the UK since the early 1980s, particularly for broadleaved trees as they offer the benefits of protection from browsing animals and sprayed herbicides, in combination with an improved micro-climate (Hart 1991). Potter (1991) demonstrated that the use of shelters consistently decreases mortality under differing conditions and with a wide number of species. In a separate study of beech, Kerr and Evans (1993a) found no significant differences in survival after three years between trees in mesh guards or shelters. It is widely believed that the greatest constraint in getting walnut trees successfully established is damage by late-spring frosts. Part of the rationale in considering shelters was therefore their ability to promote the rapid growth of trees above the normal frost level of 1.0 to 1.5 m.

There has been a limited amount of research directed towards the use of shelters with walnut. Heiligmann and Schneider (1974) found that the growth of black walnut seedlings, when exposed to a constant wind velocity (2.8 m/sec), was significantly reduced compared to those grown in more sheltered conditions (0.1 m/sec). A procedure to ameliorate such a wind influence would therefore be highly beneficial. Tuley (1983) reported that trials with *Juglans regia* showed no significant difference in height increment between trees grown in 1.2 metre shelters and those grown in open ‘guards’. No comparison with unsheltered trees was possible due to a browsing risk, and the sample size was not specified. Ponder (1991) tested 1.2 m shelters with one year-old seedlings of *J. nigra* in south-west Missouri, finding that mean height growth was significantly greater for trees within shelters compared to those with no shelters. On two out of three sites, mean heights were 18 cm greater with shelters and there was no significant difference between treatments in seedling survival. Less desirably, Ponder (1991) also reported that shelters reduced stem diameter growth and delayed hardening-off, the latter leading to large amounts of stem die-back on one site.

A trial (Alice Holt 355) was planted by the Forestry Commission in 1984 to devise optimum establishment conditions for establishing walnut including: shelters, herbicide weed control, soil cultivation, and a comparison of performance between *Juglans regia* and *J. nigra* (Forestry Commission unpublished records). Two shelters were used; a 60 cm open-net guard and a 1.2 m white 'Correx' treeshelter. The shelters improved survival and promoted greater height growth compared to the net guards. However, the effectiveness of the trial was short-lived due to poor survival (68 %), partly due to neglect and because of dubious site selection, as the soil pH was 5.1. Evans (1984) recommended that the optimum pH should be near neutral, between pH 6 and 7.

Evans and Shanks (1987) reported that three experiments of walnut planted in shelters had demonstrated that the species showed a 'good' response, graded as '2' (where 1 = very good and 5 = very poor). Potter (1991) described *Juglans regia* as one of the more suitable trees for use in shelters as they have shown more than 100 % greater mean height increment when grown in shelters compared to mesh guards, three years after planting. Snellgrove and Mayhead (1995) suggest the potential use of shelters as a means of reducing frost damage of walnut. There is no evidence for the latter, indeed, night temperatures within shelters have been shown to drop below those of the surrounding field (Potter 1991).

3.2.2 Walnut establishment trial: aims and method

The previous section highlights some problems associated with establishing a walnut plantation and some areas where further research could usefully be conducted. The following areas of original research were considered particularly important to pursue:

- the effect of varying degrees of shelter, as provided by shelters of different heights, on early growth;
- the effect of applying a stumping treatment in combination with the treeshelter treatments;
- the optimum timing of pruning operations.

The appropriate height of shelters to use for successful walnut establishment was considered an important parameter that had remained untested. The work of Pope and Mayhead (1994) and Snellgrove and Mayhead (1995), on the stumping of walnut, could be advanced by analysing the interaction of stumping with the shelter treatments. Once the trees were successfully established after two growing seasons, the optimum time for pruning operations would be tested in order to substantiate some of the recommendations in the literature. Finally, an additional aim was that this trial would highlight which practices could be applied in the establishment of the walnut

provenance/progeny trials that were to be planted within the time-scale of this research programme. Since the seeds had been so difficult and expensive to acquire, and replacement plants would not be available, successful establishment was considered very important.

The establishment trial was planted in December 1996 on former arable land at Little Wittenham, Oxfordshire, situated at 55 m above sea level and virtually free from any variation in slope. The rich alluvial soils, overlying river gravel, are classified as sandy-clay loam with the pH of 7.6 and range between 1.0 and 1.5 m deep. The trial was a randomised complete block design consisting of two 192-tree blocks, separated by 250 m (Table 3.3). Each block contained twelve 16-tree main plots representing one replicate of each of the 12 possible combinations of main treatments (Figure 3.3). Three treeshelter treatments of different heights were applied: no shelters, 75 cm shelters and 120 cm shelters. Two stumping treatments were applied: stumped and not stumped. Two sources of material, hereafter termed 'populations', were planted. The German clone 'Sämlinge' and French selected variety 'Lozeronne' (RA464), were selected for the trial because each had been developed separately in their country of origin and were likely to have diverse growing habits. From spring 1999, an additional treatment of pruning was incorporated into the trial, necessitating the splitting of the main plots into 48 sub-plots (Figure 3.3). Two pruning treatments were applied, a pruning in late February (23rd) or in late July (20th). The pruning treatment involved the removal of competing leaders and of side branches from the lower third of the trees' total stem height, as recommended by Becquey (1997).

Table 3.3 Walnut establishment trial: analysis of variance model.

	Number	Source of variation	Degrees of freedom
MAIN PLOTS	2	Blocks	1
(planted 1996)	2	Population treatments	1
	3	Treeshelter treatments	2
	2	Stumping treatments	1
		Population × Treeshelter interaction	2
		Population × Stump interaction	1
		Treeshelter × Stump interaction	2
		Population × Treeshelter × Stump interaction	2
		Main Plots Residual (error)	11
Total	24		23
SUB PLOTS	2	Pruning treatments	1
(applied 1999)		Population × Pruning interaction	1
		Treeshelter × Pruning interaction	2
		Stumping × Pruning interaction	1
		Population × Treeshelter × Pruning interaction	2
		Population × Stump × Pruning interaction	1
		Treeshelter × Stump × Pruning interaction	2
		Population × Treeshelter × Stump × Pruning interaction	2
		Sub-Plots Residual (error)	12
Total	48		47

BLOCK 1 Cpt. 303a

1 **2C** February	3 **1B*** July	5 **2C*** July	7 **2B** February
2 July	4 February	6 February	8 July
9 **1A** February	11 **1C** July	13 **2A*** July	15 **1C*** February
10 July	12 February	14 February	16 July
17 **2A** July	19 **1A*** February	21 **1B** February	23 **2B*** July
18 February	20 July	22 July	24 February

BLOCK 2 Cpt. 303b

25 **1C*** February	27 **1A** July	29 **1B*** February	31 **2B** February
26 July	28 February	30 July	32 July
33 **1B** July	35 **2C*** February	37 **1A*** July	39 **2B*** July
34 February	36 July	38 February	40 February
41 **2C** February	43 **2A*** July	45 **1C** July	47 **2A** February
42 July	44 February	46 February	48 July

PLANT MATERIAL

1 GERMAN 'Sämlinge'

2 FRENCH 'Lozeronne'

MAIN TREATMENTS

A No treeshelter

B 75cm treeshelter

C 120cm treeshelter

* Stumped to 10cm at planting

SUB PLOT TREATMENTS

February - pruning

July - pruning

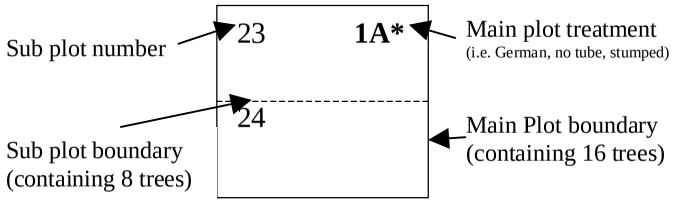


Figure 3.3 Walnut establishment trial (P96): trial design and allocation of treatments.

The trees were slot-planted at 2 × 2 m spacing. The treeshelters used were the 'Standard' manufactured by Tubex Ltd. These shelters are supplied nested in bundles and, therefore, the diameters vary from 80 to 120 mm. The stumping treatment was applied at the time of planting at 10 cm above ground level, the cut made with secateurs at 45° to aid water runoff on the cut stem.

Measurements of tree heights and stem diameters, at 7.5 cm above ground, were made at the time of planting and subsequent measurements of height were made on an annual basis. The final assessments of tree height and stem diameter at 7.5 cm were made at the end of the third growing season in 1999. Stem dieback during one year was calculated by measuring the height above ground of the highest flushing bud in the spring, this value was then subtracted from the total tree height recorded the previous autumn.

Assessments of bud and leaf flushing were made in spring 1998 using a seven point scoring system (Figure 3.4). A score of '0' was given to trees with dormant buds and thereafter 2 scores were used to record each subsequent stage; bud break (1 and 2), the emergence of leaves (3 and 4) and shoot expansion (5 and 6). A tree was awarded a particular score when approximately 50 % of the buds/leaves had attained the flushing stage. Six assessments were made over a period of 21 days in order to monitor the progression of the trees through the entire scoring system. A fully flushed state was classified as score 3; where the emerging leaves first extended beyond the bud scales.

3.2.3 Results

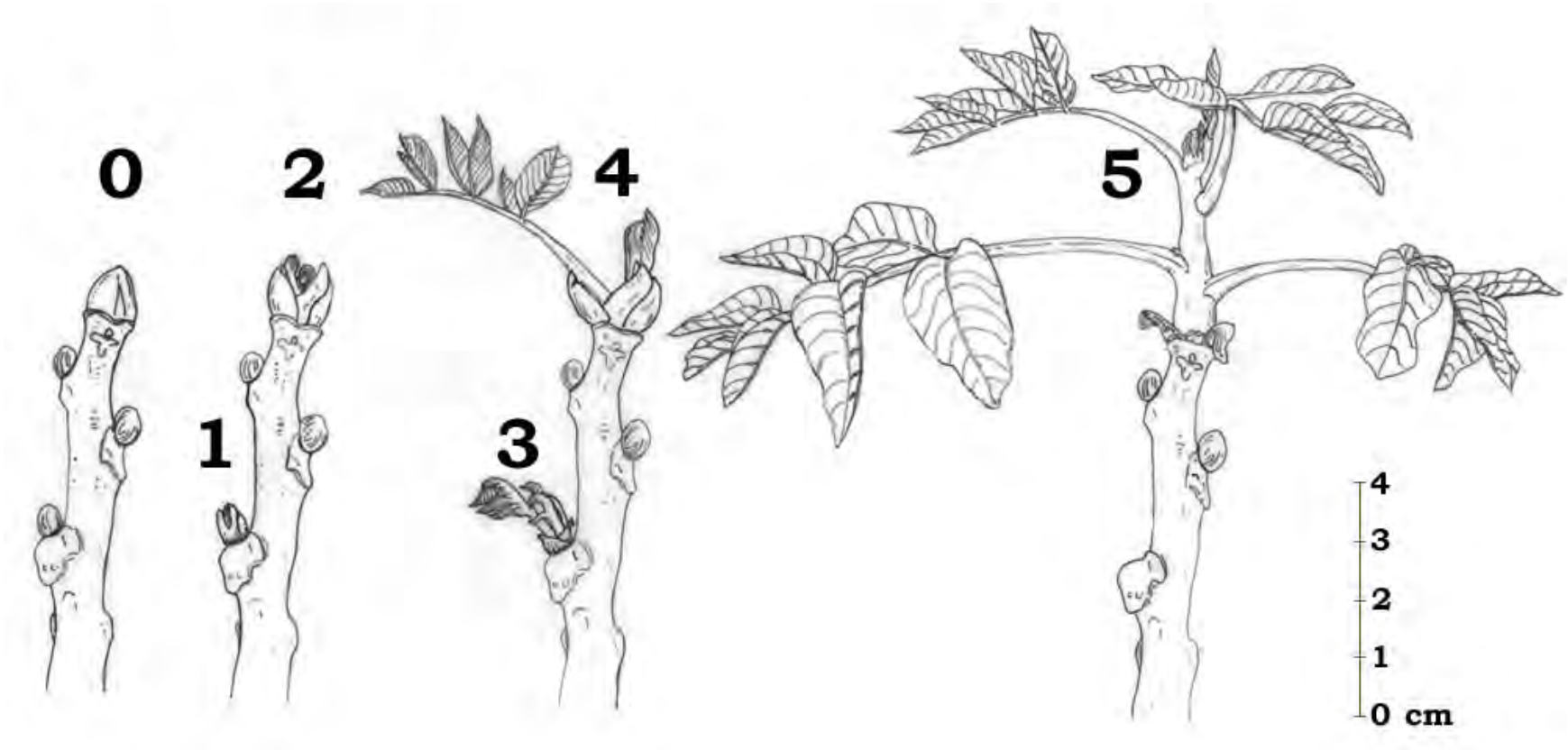
All interactions (Table 3.3) were tested but only those that were significant are reported below.

SURVIVAL

Throughout the trial, survival was excellent with a survival rate of 99.2 % after the first year (three trees were lost from a total of 384). By the end of the second year there had been a small increase in mortality with the loss of a further five trees (97.9 % survival), which remained unchanged by the end of the third year. Disparities in tree survival between all treatments were non-significant although there was variation between blocks; with 100 % survival in block 1, whereas block 2 had a survival rate of 95.8 %.

TREE HEIGHT

Total mean tree height increment over the three growing seasons was 90.4 cm. The greatest annual overall mean increment occurred in the first year with almost 50 cm of growth (Table 3.4, p.50). Height growth in subsequent years was less impressive, particularly in the second growing season, the reasons for which are explored below.



SCORE	DESCRIPTION	
0	BUD CLOSED	winter bud
1	BUD BREAKING	scales separating
2	BUD BREAKING	leaves visible
3	LEAVES EMERGING	< 2 cm (but no shoot expansion)
4	LEAVES EMERGING	> 2 cm (but no shoot expansion)
5	SHOOT EXPANDING	< 4 cm
6	SHOOT EXPANDING	> 4 cm *(not illustrated)*

Figure 3.4 Walnut flushing scores.

Table 3.4 Walnut establishment trial: summary results of tree heights and stem diameters, based on plot means. Within any one treatment and along any one line, figures followed by the same letter(s) do not differ significantly from each other at p=0.050. The significance level was calculated using ANOVA (Table 3.3) and where necessary, multiple comparison of means calculated using the Tukey test.

Assessment		Treeshelter treatments N = 8 (N = 16 from 1999)			Stumping treatments N = 12 (N = 24 from 1999)		Population treatments N = 12 (N = 24 from 1999)		Overall mean N = 24 (N = 48)
		0 cm	75 cm	120 cm	0 not stumped	1 stumped	1 Sämlinge	2 Lozeronne	
Height (cm)									
1996	mean	37.0[a]	36.4[a]	34.6[a]	62.0[a]	10.0[b]	26.1[a]	46.0[b]	36.0
	s.e.	11.9	11.5	10.3	6.1	0.0	4.9	10.9	6.2
1997	mean	45.8[a]	88.3[b]	123.4[c]	91.6[a]	80.0[b]	89.0[a]	82.6[a]	85.8
	s.e.	3.9	3.7	4.3	9.2	10.6	10.9	9.1	7.0
1998	mean	51.7[a]	105.6[b]	130.3[c]	99.7[a]	92.1[b]	97.6[a]	94.1[a]	95.9
	s.e.	3.9	3.2	3.2	9.2	11.1	10.2	10.3	7.1
1999	mean	77.8[a]	136.7[b]	164.7[c]	132.4[a]	120.4[b]	127.6[a]	125.2[a]	126.4
	s.e.	3.8	3.7	3.2	7.6	8.4	7.5	8.6	5.7
Height increment (cm)									
1996-97	mean	8.7[a]	51.9[b]	88.8[c]	29.6[a]	70.0[b]	63.0[a]	36.6[b]	49.8
	s.e.	8.4	10.1	10.7	11.3	10.6	11.6	12.2	8.7
1997-98	mean	5.5[a]	17.3[b]	6.8[a]	7.9[a]	11.9[a]	8.5[a]	11.3[a]	9.9
	s.e.	1.6	2.4	3.1	2.1	2.7	2.6	2.3	1.7
1998-99	mean	26.1[a]	30.3[a]	34.4[a]	32.1[a]	28.3[a]	30.0[a]	30.6[a]	30.3
	s.e.	2.4	2.0	1.6	1.8	1.6	1.6	1.9	1.2
TOTAL	mean	40.6[a]	100.3[b]	130.2[c]	70.2[a]	110.6[b]	101.5[a]	79.3[b]	90.4
1996-99	s.e.	7.6	6.9	6.9	9.0	8.4	8.0	10.6	6.8
Stem diameter (mm)									
1996	mean	16.9[a]	17.1[a]	17.5[a]	17.5[a]	16.9[a]	15.2[a]	19.2[b]	17.2
	s.e.	0.9	1.0	1.0	0.6	0.9	0.6	0.4	0.5
1998	mean	13.5[a]	21.4[b]	19.2[b]	19.5[a]	16.5[b]	18.0[a]	18.1[a]	18.0
	s.e.	1.3	1.1	0.5	1.0	1.4	1.1	1.4	0.9
1999	mean	21.0[a]	28.3[b]	25.4[b]	26.5[a]	23.2[b]	24.6[a]	25.1[a]	24.9
	s.e.	0.9	1.1	0.9	1.0	1.0	0.9	1.1	0.7
Stem diameter increment (mm)									
1996-98	mean	-3.4[a]	4.3[b]	1.7[b]	2.1[a]	-0.4[a]	2.8[a]	-1.1[b]	0.9
	s.e.	1.7	1.4	1.1	1.1	1.7	1.2	1.5	1.0
1998-99	mean	7.5[a]	6.8[a]	6.2[a]	7.0[a]	6.7[a]	6.7[a]	7.0[a]	6.8
	s.e.	0.4	0.6	0.5	0.4	0.5	0.4	0.5	0.3
TOTAL	mean	4.1[a]	11.2[b]	7.9[ab]	9.1[a]	6.4[a]	9.4[a]	6.0[b]	7.7
1996-99	s.e.	1.2	1.3	1.1	1.0	1.2	1.0	1.1	0.8

In the first growing season following the stumping operation (1996-97), mean height increment was significantly greater ($p<0.001$) for stumped (1) than unstumped (0) trees, the former increasing almost 2.5 times more in height than the latter (Table 3.4). Mean annual height increment remained marginally better for stumped trees during the second growing season although not significantly different from the unstumped trees. By the third growing season, the advantage had disappeared and despite the greater increment in the stumped trees, they remained smaller than the unstumped trees. In the first year following the stumping operation, there was no significant interaction between stumping and shelter treatments. Over the three growing seasons there was a significant interaction ($p=0.003$) between population and stumping treatments. Total mean height increment (1996-1999) for the German trees was 24 cm greater in the stumped treatments (113 cm) than in non-stumped treatments (89 cm), compared to a difference of 55 cm between stumping treatments for the French trees.

The heights of the two populations remained similar and non-significant throughout the three growing seasons, despite being significantly different ($p<0.001$) when planted. This was due to the significantly ($p<0.001$) greater growth of the German trees during the first growing season which evened-out the height variation between the two populations.

During the first year (1996-97), height growth was significantly ($p<0.001$) different in the shelter treatments. In plots without shelters, height growth averaged less than 9 cm but in comparison, height growth in plots with shelters was dramatic (Table 3.4). Figure 3.5 clearly illustrates that the trees in the 75 cm and 120 cm shelters had grown, on average, beyond the height of their respective shelters by the end of the first growing season in the autumn of 1997. During the next growing season, annual height increment was much poorer (Table 3.4). This may have been due to the new exposure faced by the trees as they left the protection of their shelters, as illustrated by the impressive growth of nearly 90 cm in the 120 cm shelter treatment during the first year (Table 3.4), which was apparently limited to less than 7 cm in the second year. However, an additional influencing factor was observed and quantified during the winter of 1997/98 when some trees were observed to suffer from dieback.

The assessment of stem dieback during the winter of 1997/98 produced some surprising results. Variation for dieback in the two populations was significantly ($p<0.001$) different with the German population having a 51 % greater dieback length compared to the French population. More interesting in respect to the aims of the experiment was a highly significant ($p<0.001$) effect of the shelter treatments on dieback (Figure 3.6). Mean dieback for trees without shelters and those in 75 cm shelters was 9 cm and 13 cm respectively but in the 120 cm shelter treatment,

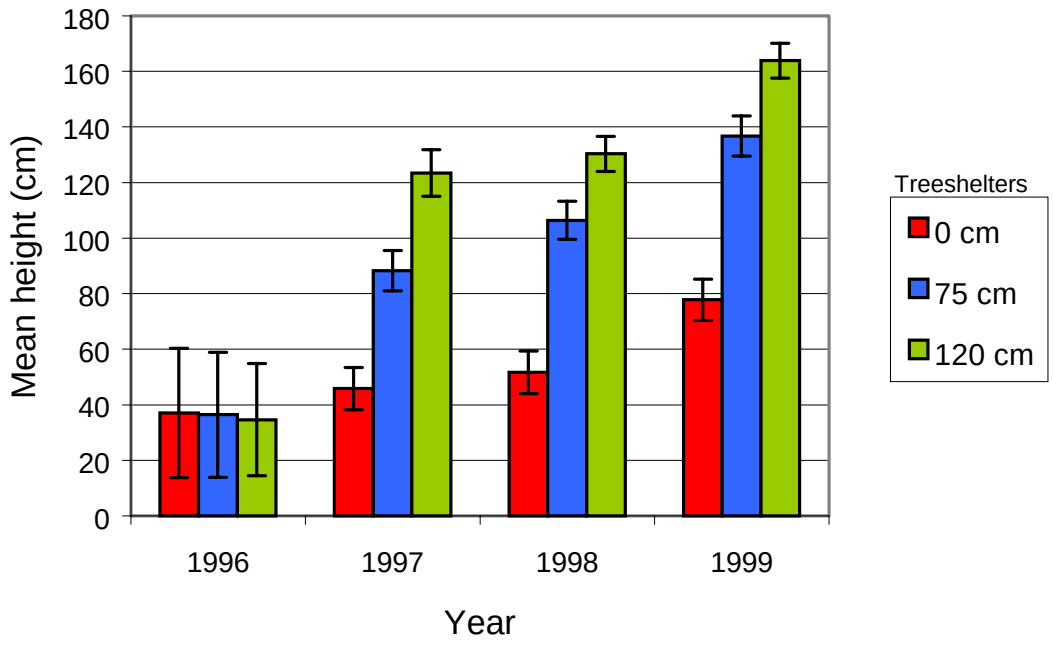


Figure 3.5 Mean end of year tree heights over three growing seasons in the treeshelter treatments of the walnut establishment trial, planted autumn 1996 (based on plot means). Error bars show 95 % confidence limits.

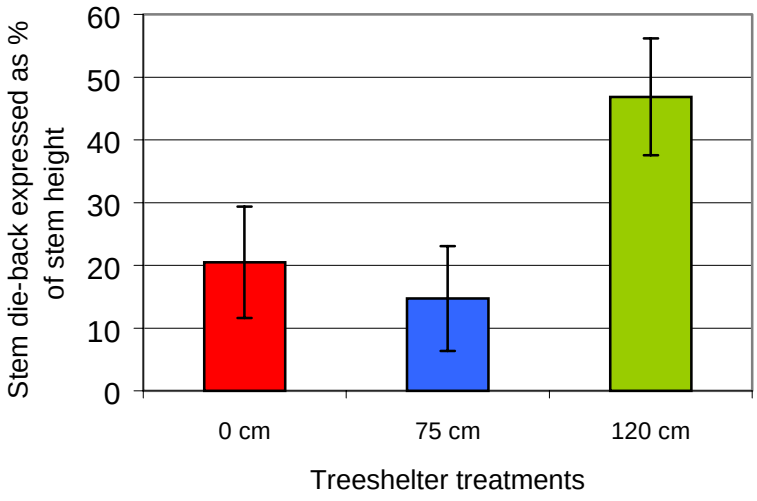


Figure 3.6 Dieback expressed as a percentage of over-winter stem height for different treeshelter treatments of the walnut establishment trial, based on plot means. Error bars show 95 % confidence limits.

mean die-back was 59 cm. A Tukey test (Hoppe 1993) confirmed those trees within tall shelters suffered significantly (p=0.001) more dieback than those in the other two shelter treatments, whilst there was no significant difference between the two latter treatments.

However, it was thought that this effect could have been influenced by tree height; *i.e.* that the taller trees were more prone to dieback. Mean over-winter tree height (1997-98) was indeed significantly different ($p<0.001$) between all shelter treatments (Figure 3.5). When dieback length is expressed as a ratio or percentage of over-winter tree height the interaction is clearer (Figure 3.6).

Mean dieback in the 120 cm shelter treatment, containing the tallest trees (123 cm), was 46 % of the trees' height. However the mean dieback ratio for trees in the 75 cm shelter treatment was actually less than for those trees without shelters, which contained significantly ($p<0.001$) smaller trees. The effect of die-back on tree height increment between 1997 and 1998 within the shelter treatments is more apparent when the mean annual tree height increment (Table 3.4), calculated by subtracting end of year heights in 1997 (H97) from 1998 (H98), is compared to the measured increment after die-back (H98 – height to highest bud in spring (*G*)) (Table 3.5).

Table 3.5 Stem dieback and its effect on estimated mean annual height increment within treeshelter treatments of the walnut establishment trial, 1997-1998.

Assessment		0 cm treeshelters	75 cm treeshelters	120 cm treeshelters
Calculated height increment (H98-H97) (cm)	mean	5.5	17.3	6.8
	s.e.	1.6	2.4	3.1
Post die-back height increment (H98-*G*) (cm)	mean	14.5	30.5	65.9
	s.e.	2.7	4.1	5.0

Post die-back increment was significantly ($p<0.001$) different between all shelter treatments and growth was substantial in the 120 cm shelter treatments compared to the others. During this one season, die-back therefore had a significant effect on height growth within the 120 cm shelter treatments, as the average growth was 66 cm but this followed an over-winter die-back of 59 cm (Figure 3.7). One pertinent question is whether the substantial increment in the 120 cm shelter treatment would have occurred if die-back had not preceded it? This may be answered by considering that the mean highest flushing bud (*G*) in the 120 cm shelter treatment was at 64.4 cm (s.e. = ±5.8), or only half the height of the protecting tree shelters. The mean growth of 66 cm that followed during 1998 was therefore almost entirely within the cover of the 120 cm shelters. In contrast, the mean height of *G* in the 75 cm shelter treatments was 75.1 cm (s.e. = ±4.7) and the 30 cm of growth that followed took place above the protection of the 75 cm shelters.

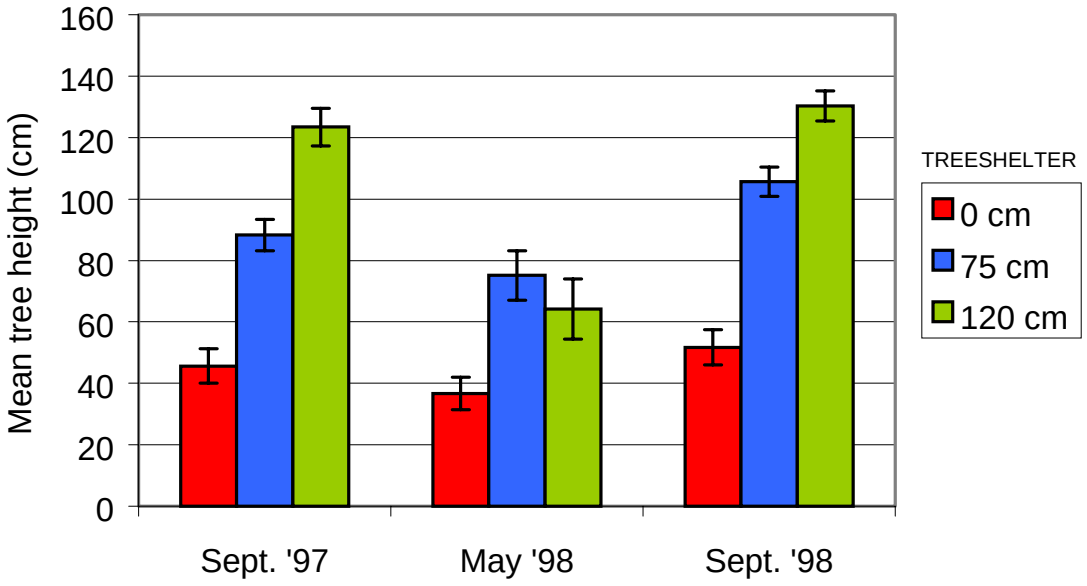


Figure 3.7 Mean tree heights between 1997 and 1998 in the treeshelter treatments illustrating over-winter die-back (based on plot means). The September assessments indicate mean end of year tree heights whilst the May assessment indicates the mean height of the highest flushing bud. Error bars show 95 % confidence limits.

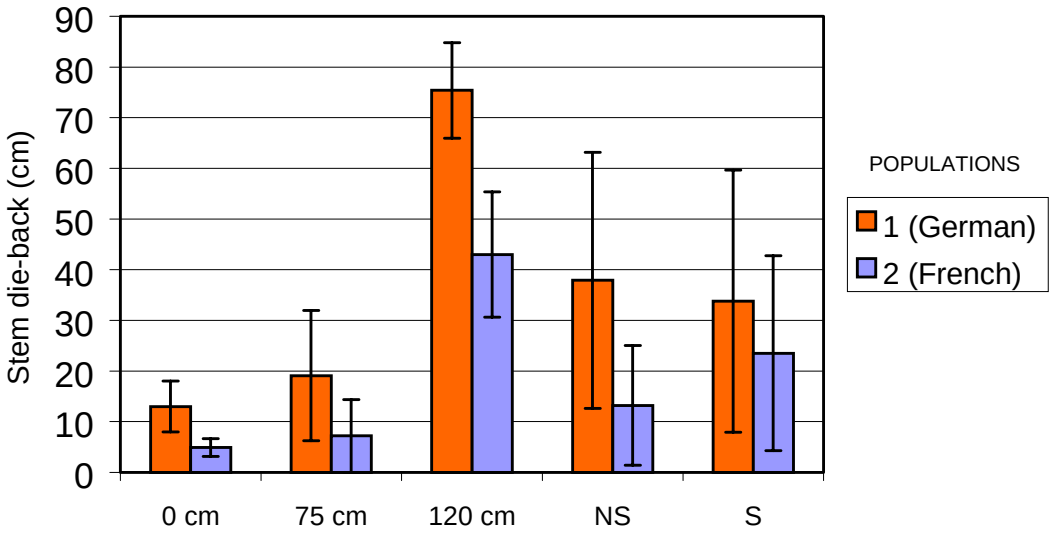


Figure 3.8 Mean stem die-back for the two populations in the treeshelter (0 cm, 75 cm and 120 cm) and stumping treatments (NS = not stumped and S = stumped), based on plot means. Error bars show 95 % confidence limits.

There were no significant differences in the stumping treatments for stem die-back. However, the two populations suffered significantly (p<0.001) different degrees of stem die-back; the German trees suffering an average die-back of 36 cm, twice that of French trees whose mean die-back was

18 cm. The two populations also showed significant interactions with the shelter treatments (p=0.025) and with the stumping treatments (p=0.050) (Figure 3.8). The French trees had greater die-back when stumped than when not stumped, although they was still less afflicted than German trees in either stumping treatment.

Height growth during the third growing season was more favourable than in the previous year, with a total mean increment of 30 cm (Table 3.4). Die-back was absent from all the treatments during 1999. Tree heights within the shelter treatments remained significantly different (p<0.001) although height increment during the third growing season was not significantly different between them. Total height and annual increment in the other treatments during the third growing were all non-significant (Table 3.4).

The total height increments over the three growing seasons (1996-99) were significantly different (p<0.001) for all treatments (Table 3.4). For the stumping and population treatments, however, these can be explained by significant (p<0.001) differences in height increment in the first year (1996-97) only because thereafter there were no significant differences for either treatment.

STEM DIAMETER

Stem diameters were significantly (p<0.001) different between the two populations at planting time. At the next assessment of stem diameter, after the second growing season in 1998, population differences were non-significant. However there was a significant (p<0.001) variation between shelter treatments. A multiple comparison of plot means using the Tukey test (Hoppe 1993) confirmed that differences between the 75 cm and 120 cm shelter treatments were non-significant but that the diameters in these two treatments were significantly (p=0.050) greater than those in the unsheltered trees (Table 3.4). Differences in mean stem diameters of the two stumping treatments were non-significant.

Stem diameter increments between 1996 and 1998 were significantly different within the shelter treatments (p=0.001) and population treatments (p=0.007) but just non-significant (p=0.057) for the stumping treatments (Table 3.4). A surprising result over the two growing seasons was that mean diameters for some treatments showed negative growth. This reduction in mean stem diameter was due to die-back of the original stem which was then replaced by numerous shoots, each of a smaller diameter.

Total mean stem diameter by the end of the third growing season in the autumn of 1999 was almost 25 mm, after a total mean increment of 7.7 mm over the three growing seasons (Table

3.4). Stem diameters at the end of 1999 were significantly different between shelter treatments ($p<0.001$) and stumping treatments ($p=0.014$) but non-significant between the two populations. Over the three growing seasons the 75 cm shelters promoted significantly ($p=0.007$) greater stem diameter growth than the control treatment (Table 3.4).

The relationship between shelter treatments and stem diameters was explored further at the end of 1999. Those trees that had grown sufficiently were also measured for diameter at breast height (*dbh*) at 1.3 m and ground level (*dgl*). Subplot means were then estimated, although subplots with fewer than five trees of minimum 1.3 m in height were excluded, for *dbh*, stem volume and stem taper. Subplots means were not available for the 'no shelter' treatments but for the 75 cm and 120 cm shelter treatments, there were 6 and 14 subplot means respectively. The lack of so many subplots restricted the use of a full linear model in any analysis and so any conclusions at this time must be evaluated with caution. Stem volume was calculated by the following equation for a linear truncated cone:

$$V = \frac{\pi}{12}(dgl^2 + dbh^2 + (dgl \times dbh))L$$

where V = volume (cm^3), *dgl* = diameter at ground level (cm), *dbh* = diameter at breast height (1.3m) (cm) and L = length (cm).

Stem volumes were calculated for stem length between ground level and 1.3 m. Trees in the 75 cm shelters had attained a mean volume of 582.2 cm^3 (s.e. ±42.2) which was greater than trees in the taller 120 cm shelters which had a mean volume of 387.3 cm^3 (s.e. ±32.5). However, the large standard errors suggest that these two estimates should not be viewed as being significantly different.

Stem taper, expressed as a coefficient of change in diameter in respect to unit of length, was calculated using the following formula:

$$\frac{dgl - dbh}{L}$$

where *dgl* = diameter at ground level (cm), *dbh* = diameter at 1.3 m (cm), L = length (cm)

Mean stem taper was greater for trees in the 75 cm shelters than for those in the 120 cm shelters. Calculated 95 % confidence limits for stem taper did not overlap for the two shelter treatments. For the 75 cm treatment, for every cm in length, stem diameter changed by 0.18 mm (s.e. ± 0.005). In other words, along the 130 cm between the *dgl* and *dbh* measurements, the total

difference between mean *dgl* and *dbh* in 75 cm shelters was 2.34 cm (0.01807 × 130). In the 120 cm treatment stem taper was 0.11 mm (s.e. ± 0.005) for every cm in length, equating to a mean difference between *dgl* and *dbh* of 1.45 cm. The difference in stem taper between the two treatments is explained by the larger mean *dgl* measurements for trees in 75 cm shelters compared to 120 cm shelters (Table 3.4). At breast height there were marginal differences in stem diameter, although significantly different (non-overlapping 95 % confidence intervals), between the two treatments, with trees in the 75 cm shelters having a smaller mean *dbh*.

FLUSHING ASSESSMENTS

Analysis of flushing was undertaken using the Kruskal-Wallis non-parametric test on each of the assessment dates. As expected, there were no significant differences in flushing dates within the stumping treatment. On the first two assessment days, Julian days 125 (May 5th 1998) and 128, there was significant (p=0.050) variation between the median flushing scores for the two populations. German trees had flushed earlier than French trees by a mean score of 0.8 (s.e. ±0.1) on day 125 and remained 0.7 greater (s.e. ±0.2) on day 128. From day 131 onwards, variation between the two populations was non-significant.

There were some clear trends in flushing within the shelter treatments, as revealed in Figure 3.9. The 120 cm shelter treatment promoted earlier flushing, and indeed those trees in the tall shelters reached the 'flushed' stage (score 3), approximately five days before either of the other two treatments. Differences between the median values for the three shelter treatments were significant at p=0.020 on the earliest assessment day (day 125) and highly significant (p<0.001) thereafter. In order to clarify which treatments were different on each assessment day, multiple statistical comparisons were applied with the test statistic Q ([5]). On the first (day 125) and last (day 146) assessment days, there were no significant differences between the treatments. On days 128, 131, 134 and 138, flushing in the 120 cm shelters was significantly (p=0.050) more advanced than in the other two shelter treatments. Differences between the no shelter and 75 cm shelter treatments were non-significant except on days 134 and 138 (p=0.050).

[5] The test statistic Q is derived from the z statistic (used in the z test for comparison between 2 populations) but uses a more stringent criterion for rejection because of the multiple comparisons being undertaken.

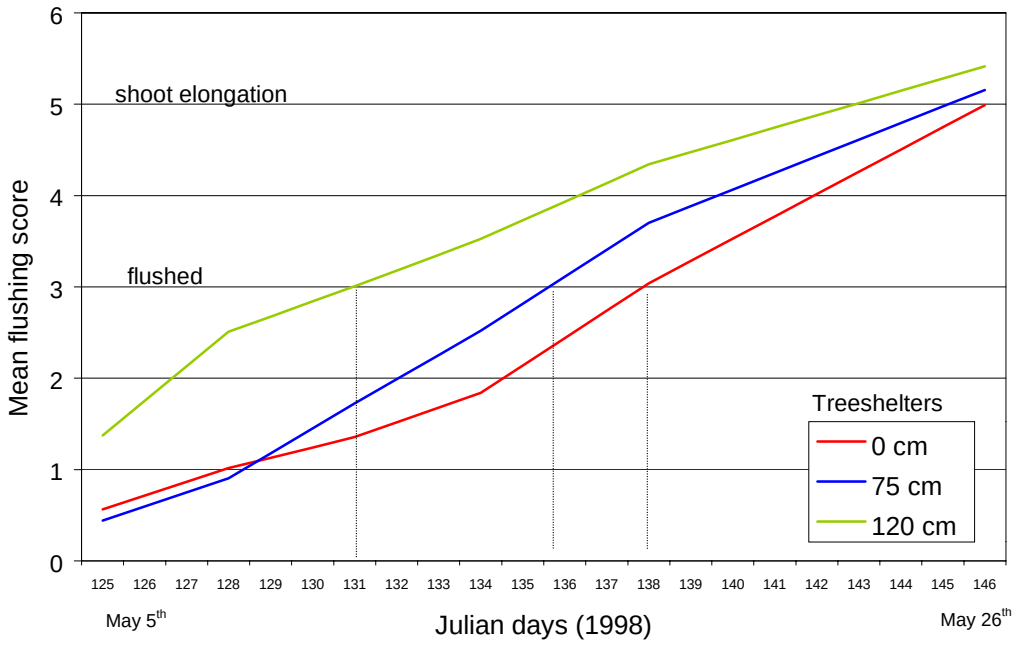


Figure 3.9 Progression of flushing in the treeshelter treatments in 1998 (based on plot means). Vertical dotted lines indicate the day on which a mean score of three ('flushed') was attained by each shelter treatment.

PRUNING TREATMENTS

The two pruning treatments, although only undertaken during the final assessment year in the establishment trial, highlighted a number of interesting effects on tree growth. Mean tree heights at the end of the 1998 growing season, prior to the initiation of pruning, were not significantly different between the sub-plots to be pruned. By the end of 1999, total mean tree heights were significantly (p=0.030) greater in February pruned trees than in July pruned trees, although the difference in mean heights was only 6 cm.

The pruning treatments had a significant (p=0.003) effect on stem diameters as the mean diameter of the February-pruned trees increased to 26.1 mm by the end of 1999, compared to 23.7 mm for July-pruned trees. Increment of stem diameters was also significantly (p=0.005) different between the two pruning treatments. This early result is similar to the findings of Falcioni and Buresti (1997), where winter (February) pruned trees in Italy had greater diameter growth over four years than autumn (October) pruned trees.

A notable, if predictable, effect of the pruning treatments was on tree form with the February-pruned trees having more branches, and in some cases more competing leaders, than those pruned in July. This was a natural consequence of the fact that the trees pruned in February had nine

months to develop post-pruning, whilst those pruned in July had only two months of growth, before the trees were measured in September. In effect, trees in the pruning treatments had not yet undergone a full year of development before assessments were made. Formal assessment of tree form was not therefore undertaken partly for this reason, and because of the difficulty in making accurate or objective assessments of tree form at such a juvenile stage.

3.2.4 Discussion and Conclusions

The shelter treatments had the strongest influence on tree growth in the establishment trial. Compared to trees planted without the protection of a shelter, those in shelters had markedly improved growth rates in the first two years after planting. For walnut growers, the obvious recommendation would therefore be to use shelters wherever possible. However, the greater height increment observed in the 120 cm shelter treatment was limited to growth within the confines of the shelters. This factor, in combination both with the high degree of die-back observed in the 120 cm shelters, and the promotion of earlier flushing, counts heavily against their use for the successful establishment of walnut. The 75 cm shelters provided greater protection from die-back than the 120 cm shelters whilst not significantly increasing this risk in comparison to the unsheltered treatment. Height growth for the unprotected trees was consistently poorer than the other shelter treatments. The practical recommendation to walnut growers would be to use the smallest sized shelter possible, given the need to protect against browsing; *i.e.* 0.6 m for rabbits, 0.75 m for hares and 1.2 m for muntjac and roe deer (Potter 1991).

Stem diameter growth within the shelter treatments was similarly variable, although the recording and assessment units (millimetres) were very small, suggesting that some caution should be used in comparing closely matching results. The results do, however, point convincingly to the advantage of the 75 cm shelters, over both taller shelters and no shelters. Calculation of stem taper indicated the columnar nature of trees in the 120 cm shelter treatment. This less stable form may explain the observed tendency for these trees to be unable to support their own weight, relying on the strength of the shelter's supporting stake. The use of tall shelters on exposed or windy sites, where the stakes are subjected to considerable forces in addition to the weight of a weak tree, should therefore be avoided if possible.

The observations of die-back within the trial highlighted an unexpected correlation with the shelter treatments. An explanation for this could be the effect of the shelters on stem hardening at the end of the growing season. Artificially high temperatures within the shelters, as have been reported by Potter (1991) and Rendle (1985) may delay lignification. A study of leaf-fall or lignification within the shelter treatments may clarify this question, which unfortunately was not

possible within the confines of this study. The young trees in the shelters may have been damaged by temperature extremes within the tubes during the dormant season. Rendle (1985) reported that daytime temperatures within 1.2 metre shelters during February to April were higher than in the surrounding field whilst at night, temperatures within the tubes dropped below those in the field. For example, on one sunny day in March, Rendle (1985) recorded temperatures ranging from –2.2 to +23.8 °C within shelters, whilst field temperatures ranged from –2.2 to only +11.3 °C. Stems unprotected by shelters therefore remain at a relatively stable temperature, whilst those inside them may, during certain conditions, be subjected to repeated freeze-thaw cycles during sunny winter days and cold nights.

The stumping treatment applied in this trial produced results similar to those reported in other studies, *e.g.* Evans (1984) and Snellgrove and Mayhead (1995), in that it promoted rapid height growth in the year following the operation. Shelters had no effect on tree height or diameter in combination with the stumping operation. The high mortality rate caused by stumping, reported by another study (Forestry Commission unpublished), did not occur in this trial. The rapid height increment in the first year following stumping would be advantageous if this growth took the tree beyond the frost-prone zone (0.0 to 1.5 m), as suggested by Pope and Mayhead (1994). In this trial however, although height increment was impressive, it failed to reach the end of season height attained by the unstumped trees. There is no evidence from this research that the practice of stumping is advantageous because neither tree height nor stem diameter of stumped trees was greater than among the unstumped trees.

The pruning operation introduced in 1999 has indicated that the timing of pruning walnut trees is an important area for further research. Assessments of this treatment will continue for at least the next ten years in order to gauge any long-term effects with the aim of giving the walnut grower reliable advice.

3.3 Crown and stem diameter relationship

3.3.1 Introduction

Forest trees usually exhibit a significant correlation between their crown and stem diameters which can be used for predicting basal areas, devising thinning regimes and, given the inclusion of height data, for developing stand volume estimates. Duchaufour (1903) was one of the first authors to identify a linear relationship between crown diameter (*cd*) and stem diameter (*d*) in his study of *Fagus.* Subsequently workers have identified the *cd:d* relationship for many species both temperate and tropical, coniferous and broadleaved. Dawkins (1963) concluded that the most practical interpretation of this relationship is that it is linear up to the typical rotation age of a crop, based on work with 17 tropical tree species, giving a linear equation of the form:

$$\text{crown diameter} = a + (b \times \text{stem diameter})$$

The true relationship between *cd* and *d* may actually be sigmoid due to the distortion of the line at the lower end because *d* is usually measured at breast height, and the possible depression of the upper end due to senility (Dawkins 1963). However, Dawkins (1963) proposed that for the common range of forest tree sizes, between 0.2 and 0.5 m *dbh*, there would be negligible distortion of the linear relationship.

Dawkins (1963) also illustrated how the *cd:d* relationship for a number of tropical tree species could be used to indicate the tolerance of a species to stand density. Crown and stem diameter relationships have been used to calculate maximum basal areas for species, such as beech (Colette 1951) and Douglas Fir (Briegleb 1952), and for predicting desirable spacings or stocking rates for others such as ash, cherry and sycamore (Thill 1980).

Stand density indexes (*e.g.* Curtin 1964) and crown competition factors (*e.g.* Krajicek *et al.* 1961) have been calculated for a number of species. These estimates supply an indication of the area available to trees for stands at any particular density. More recently, this theory has been extended by other workers to produce three dimensional crown taper models. Warbington and Levitan (1992) propose that crown taper models can be applied for estimating wildlife habitat relationships for forest-dependent species, as the amount of canopy cover at different heights determines the suitability of a stand as habitat for wildlife.

Many workers, *e.g.* Ilvessalo (1950), Ayhan (1974), Gering and May (1995), have used the crown:stem diameter relationship of species for estimating *dbh* or basal area from crown diameters measured from aerial photographs.

3.3.2 Crown and stem diameter relationship for *Juglans regia*

There are no published *cd:d* equations for *Juglans* species, although Savill (1998) reported that crown and stem measurements were taken from a number of trees near Oxford. Walnuts with a 60 cm *dbh* were predicted to have a crown diameter of 13.4 m, which is wider than most forest trees of the same diameter (Savill 1998). An enhanced understanding of this relationship was viewed to be an important parameter, not only to further silvicultural understanding but more specifically, to decide the spacings to be used in the provenance/progeny trials (Chapter 5). Measurements were made of the crown and stem diameters of 50 open-growing trees in England and 70 trees from the walnut forests in Kyrgyzstan (Chapter 2) and regressions calculated.

The walnut trees in the Kyrgyz forests were growing in heterogeneous environmental conditions and often on precipitous slopes making accurate measurement difficult. Breast height diameters ranged from 13 to 128 cm and crown diameters from 5 to 19 m (Section 2.2). The regression equation for the Kyrgyz walnuts was:

$$y = 5.24 + 12.1x \qquad r^2 = 73.9\ \%$$

where y is the crown diameter and x is the stem diameter. Although the *cd:dbh* relationship was significant ($p<0.001$) it had a relatively low value of r^2, and a higher intercept (a) value (5.24), compared to many other tree species (Table 3.7, p.64). Consequently, the calculated regression was judged unreliable, probably attributable to the problems of accurate measurement.

The regression calculated from the English open-grown trees indicated a much stronger relationship (Tables 3.6 and 3.7). The intercept (a) at 2.71 in the calculated regression is still high when compared to those calculated for other tree species, both temperate (Table 3.7) and tropical. Dawkins (1963) describes a regression line with a positive value for a, as the most common for western North American and European species, and suggests that this type of relationship should allow a tree crop to mature as predicted by yield tables.

Dawkins (1963) stated that the variability of the intercept (a) within a species is related to the sensitivity or tolerance of the crown to competition but probably not to shade tolerance. Where a varies little or remains near zero, the species is described as 'unplastic' in crown diameter and intolerant of crowding. Conversely for 'plastic' or tolerant species, the magnitude of a indicates the intensity of crowding and a high positive a is taken to indicate excessive density (Dawkins 1963). Following this definition *Juglans regia* would be viewed as a plastic species and therefore tolerant of crown competition. However, it is commonly stated that, in plantation forestry, *J. regia* is a light demanding species, which needs to be open grown (Klemp 1979, Evans 1984, Becquey 1997). Indeed, when the species listed in Table 3.7 are classified according to their light

requirements or shade tolerance (Hart 1991) there seems no obvious clarification of this relationship with *a*.

Table 3.6 Regression equation and analysis of variance for crown and stem diameter relationship of *Juglans regia*, based on 50 open-growing trees in England.

Regression equation

$$\boldsymbol{y = 2.71 + 17.6x}$$

where $y = cd$ and $x = dbh$ (all units in metres)

Predictor	Coef	StDev	*T*	*p*
Constant	2.7063	0.2301	11.65	0.000
x	17.5688	0.5297	33.17	0.000

$S = 0.9129$ $r^2 = 96.4\ \%$

Analysis of Variance

Source	*df*	*SS*	*MS*	*F*	*p*
Regression	1	917.01	917.01	1100.27	0.000
Error	41	34.17	0.83		
Total	42	951.18			

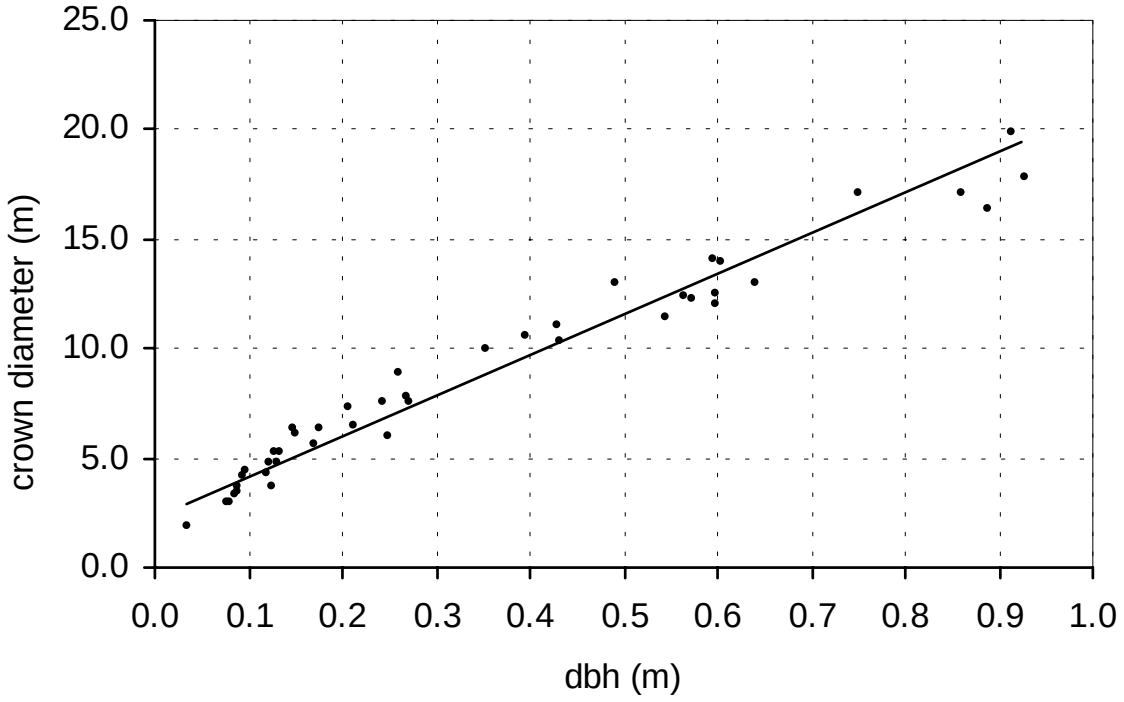


Figure 3.10 Regression plot of crown and stem diameter (*dbh*) for *Juglans regia*, based on 50 open-growing trees in England.

Table 3.7 Regression parameters for crown and stem diameters of major broadleaved tree species used in UK forestry.

Species	intercept (*a*)	slope (*b*)	r^2	Light requirement [1]
Castanea sativa Mill [2]	2.7915	10.67	0.76	***
Juglans regia	2.7100	17.60	0.96	***
Prunus avium L. [3]	1.7600	15.40	0.84	***
Betula spp. [2]	0.9563	16.19	0.92	***
Fagus sylvatica L. [2]	0.9510	15.73	0.94	*
Fraxinus excelsior L. [2]	0.7590	18.90	0.93	***
Quercus petraea (Matt.) Liebl. and *Q. robur* L. [2]	0.7328	17.72	0.95	***
Acer pseudoplatanus L. [2]	0.5930	18.90	0.82	**

Light requirement [1] denoted by * low, ** intermediate, and *** high, adapted from Hart (1991).
Sources: [2] Savill (1991), [3] Pryor (1985).

3.3.3 Basal areas

Dawkins (1963) described a simple method of estimating basal area per hectare (G m^2 ha^{-1}), from *cd*:*dbh* regression equations:

$$G \text{ m}^2 \text{ ha}^{-1} = \frac{10^4 \times 0.7854}{z^2}$$

where 10^4 is the area of 1 ha in m^2, 0.7854 is the canopy density for circular touching crowns at square planting and z = the crown diameter: stem diameter ratio (*cd/dbh)*. The maximum number of trees per hectare (N) is calculated as:

$$N = \frac{10^4}{cd^2}$$

These calculations were applied to the walnut regression equation for stem diameters from 0.10 to 0.70 m at breast height (Table 3.8). The *cd:dbh* ratio values are seen to diminish by more than 50 % from 44.70 to 21.47. Such a reduction is at variance with the results of studies based on other species . For example, the regression equation for *Fraxinus excelsior* from Savill (1991), was submitted to the same calculations and produced a *cd:dbh* ratio which ranged from 26 to 20 at 0.10 and 0.70 m *dbh* respectively. Savill (1991) stated that most trees maintain an almost constant *cd:dbh* ratio throughout the important stages of the silvicultural rotation (0.20 to 0.50 m *dbh*). For walnut between 0.20 and 0.50 m *dbh*, the *cd:dbh* ratio varies from 31 to 23

respectively, and between 0.10 and 0.25 m *dbh*, the *cd:dbh* ratio reduces by 64 %. The *cd:dbh* values for ash between 0.10 and 0.25 m *dbh*, using the regression calculated by Savill (1991), indicate a 17 % reduction. To illustrate the differences of *cd:dbh* ratios between walnut and some other species, the regression equations listed in Table 3.7 were used to calculate *z* values for each species and these were plotted against *dbh* (Figure 3.11). When the *cd:dbh* ratios are plotted the variation between different species is apparent, particularly at smaller stem diameters (< 20 cm *dbh*), at which point the regression is less reliable, as predicted by Dawkins (1963). Sweet chestnut follows a similar pattern to walnut in that it has a high initial value for *z* (and for *a*) but proceeds to reduce with stem diameter increment, dropping by 62 % from 0.10 to 0.70 m *dbh*. Walnut drops by less (52 %) in the same range, and is followed by cherry (46 %).

Basal area accumulation for walnut (Table 3.8) is lower compared to standard yield class tables for any other species. At 60 cm *dbh*, the basal area for walnut is 16.1 m^2 ha^{-1} whilst for oak and ash (Edwards and Christie 1981) it is 22.5 and 36.4 m^2 ha^{-1} respectively. Cherry at 59cm *dbh* was calculated to have a basal area of 31 m^2 ha^{-1} (Pryor 1988). The low basal area accumulation for walnut is an obvious consequence of the comparatively low initial stocking rates (500 trees ha^{-1}) that are proposed here (Table 3.8).

Table 3.8 Stand density and basal area for walnut *Juglans regia* stands based on 100 % canopy closure and square planting.

dbh (m)	*cd* (m)	*z* *cd:dbh* ratio	*N* trees/ha	Basal area (G m^2 ha^{-1})
0.10	4.47	44.70	500	3.9
0.15	5.35	35.67	349	6.2
0.20	6.23	31.15	258	8.1
0.25	7.11	28.44	198	9.7
0.30	7.99	26.63	157	11.1
0.35	8.87	25.34	127	12.2
0.40	9.75	24.38	105	13.2
0.45	10.63	23.62	88	14.1
0.50	11.51	23.02	75	14.8
0.55	12.39	22.53	65	15.5
0.60	13.27	22.12	57	16.1
0.65	14.15	21.77	50	16.6
0.70	15.03	21.47	44	17.0

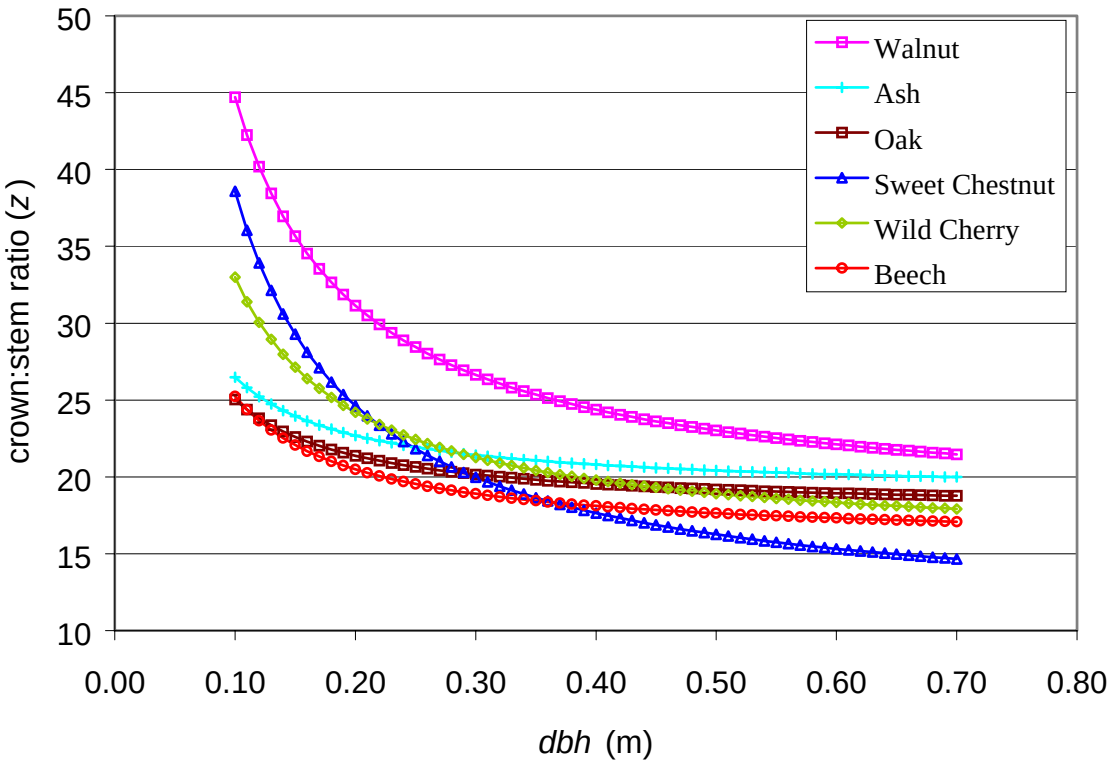


Figure 3.11 Crown diameter: stem diameter ratios (*z*) against *dbh* for six broadleaved species.

3.3.4 Thinning regimes

The regression equation for *cd:dbh* of a species can be used to calculate a 'thinning regime' which ensures that the relationship between basal area growth and the number of stems per hectare remains within realistic limits (Philip 1994). A thinning regime for *Juglans regia* was calculated (Table 3.9) which summarises thinning regimes based on a mean stem diameter to be achieved, rather than the typical age-dependent tables.

An estimate of stem volume has been incorporated as an indication of productivity although care should be taken in using these preliminary figures. The volumes in Table 3.9 were calculated using a function developed by Crockford (1987) for estimating volumes from coppice woodland, and used later by Corbyn *et al.* (1988) for estimating the branchwood and stemwood components of oak, ash and beech. Corbyn *et al.* (1988) found that the various components of volume could be predicted accurately for the three species based on *dbh* alone; for ash the coefficient of determination (r^2) was 0.976. The best fit functions of the predictive models were tested by reference to their Furnival Indices; described by Furnival (1961) as the 'average standard error transformed to the units of volume, calculated via the geometric means of the dependent variables'. Corbyn *et al.* (1988) stated that the inclusion of height in the regressions often added little to the precision of estimates, and in some cases decreased accuracy.

Table 3.9 Thinning regimes for walnut based on 100 % canopy closure and square planting. (For method of preparation, see main text).

Stem Diameter	MAIN CROP after Thinning				Yield from THINNINGS			CUMULATIVE PRODUCTION
dbh cm	Equivalent square spacing m	Number of trees per ha	Basal Area $m^2\ ha^{-1}$	Volume to 7 cm top diameter $m^3\ ha^{-1}$	Number of trees per ha	Mean volume per tree to 7 cm top diameter m^3	Total volume to 7 cm top diameter $m^3\ ha^{-1}$	Volume to 7 cm top diameter $m^3\ ha^{-1}$
20	6.2	258	8.1	56.3	142	0.22	31.1	87.5
25	7.1	198	9.7	72.3	60	0.37	21.9	125.3
30	8.0	157	11.1	97.8	41	0.62	25.7	176.5
35	8.9	127	12.2	126.5	30	0.99	29.4	234.6
40	9.8	105	13.2	155.4	22	1.48	32.4	295.8
45	10.6	88	14.1	183.3	17	2.07	34.6	358.3
50	11.5	75	14.8	209.6	13	2.78	36.1	420.7
55	12.4	65	15.5	234.1	10	3.59	37.2	482.4
60	13.3	57	16.1	256.8	8	4.52	37.8	542.9
65	14.2	50	16.6	277.8	7	5.56	38.1	602.0
70	15.0	44	17.0	297.3	6	6.71	38.1	659.6

From the volume functions developed by Corbyn *et al.* (1988), the function for ash was selected here as this species is light-demanding and fast growing and therefore likely to be more similar to *Juglans regia*, than oak or beech:

$$V\ m^3 = 0.74701 - 0.071089d + 0.0022335(d^2)$$

where V is the volume of a tree to 7 cm top diameter over bark and d is stem diameter.

The single tree volumes for walnut trees with 40 and 60 cm stem diameters (Table 3.9) were compared with single tree volumes predicted in standard yield class tables (Edwards and Christie 1981). At 40 cm *dbh* the single tree volumes for walnut are within 1 % of those for 40 cm ash and 12 % for 40 cm oak, whilst at 60 cm *dbh*, within 63 % of values for ash and 17 % for oak. Single tree volumes for wild cherry (Pryor 1988) are within 1 % of those for predicted here for walnut at 40 cm *dbh*, and at 49 cm *dbh* (the largest diameter listed), within 24 %.

Cumulative production volumes are low for walnut compared to those predicted for many other species. This is a consequence of the wide spacing required that in turn has affected basal area increment.

3.3.5 Discussion and conclusions

The crown diameter: stem diameter relationship for walnut calculated here, has provided a means of predicting stand densities, basal areas and 'thinning regimes' in walnut plantations. As no other research in this field has been conducted previously for *Juglans regia*, accurate validation of these predictions is impossible without extensive additional fieldwork.

The regression of *cd:dbh* indicates that a plantation of *Juglans regia* should be maintained at a lower density than other broadleaved timber species, as recommended by Evans (1984) and Becquey (1997). Consequently, cumulative volume production is low compared to other species. For a walnut grower, the balance between providing adequate light and a sufficient number of stems to promote vertical stem growth is important. Klemp (1979) suggested that walnuts should be maintained in competition-free conditions, from 30 to 40 years old onwards, to promote the production of large stem diameters. This silvicultural technique is often referred to as 'free-growth', which is defined as; 'a system which stimulates vigorous crown development of selected trees, in order to achieve maximum radial stem increment' (Jobling and Pearce 1977). Early research with oak by the Forestry Commission (Hummel 1951) demonstrated that timber height of open-growing oak was usually low compared to oak grown in high forest, making green pruning essential, although the rate of stem radial growth was substantially faster in the free-grown oak. Consistent with the planting densities proposed here, Jobling and Pearce (1977) stated that a very small number of young trees are sufficient to achieve complete stocking at maturity. Free-growth in oak is a recommended practice when the trees have reached the pole stage, at least 12 m in height, and are sufficient in density (Jobling and Pearce 1977). More recent data from experiments with free-grown oak (Kerr 1996), generally support the positive benefits reported by Hummel (1951), namely greater crown diameter, stem diameter and individual tree volume production for free-grown trees than for trees in high forest. Kerr (1996) concluded that the practice was still uncommon in Britain, mostly due to the cost of controlling epicormic shoots in oak. He proposed that free-growth regimes could be applied with more advantage to other broadleaved tree crops such as ash (*Fraxinus excelsior*), sycamore (*Acer pseudoplatanus*) and wild cherry (*Prunus avium*), because they can be grown on relatively short rotations. Indeed, there is some evidence that this regime may be successfully applied to walnut. Clark (1967) reported that black walnut (*J. nigra*) aged 45, when given complete crown release, grew twice as fast as unreleased trees during the four years following release.

Access to height and age data would have allowed the production of a yield table for walnut in conjunction with the above thinning regime. Without these data however, the predictions cannot be advanced any further without the use of stem analysis, which was not attempted. Limited details exist in the literature regarding annual rates of height and stem increment. Evans (1984)

stated that *Juglans regia* may have an annual stem diameter increment of 1 cm on a good site. This is supported by Becquey (1997) who stated that in similar conditions, annual stem circumference increment of 3 cm (0.95 cm diameter) is possible. Crawford (1996) suggested that the height growth rate for walnut should be approximately 40 cm year $^{-1}$. Three-year results from the walnut establishment trial (Section 3.2) indicated a mean annual height increment of 30 cm during this early phase of establishment.

The calculation of the crown and stem diameter relationship for *Juglans regia* has provided an important indication of a suitable spacing in the establishment of the provenance/progeny trials (Chapter 5). A spacing of 5 × 5 m at planting time should provide sufficient space for the trees to grow for 15 to 20 years, based on an estimated stem diameter increment of 1 cm year^{-1}, before crown competition begins. This should provide sufficient time for important assessments to be made on the trees' performance before intervention becomes necessary and selections are made.

The comparisons of the single tree volumes produced in Table 3.9, with those for other species predicted from yield tables, provide a useful indication that the volume estimates presented here are likely to be reasonably reliable, particularly for stem diameters (*dbh*) up to 40 cm. Further validation of the volume estimates for walnut would require an independent study of single tree volumes. It is hoped that the data presented here will provide a useful platform from which other workers may launch more detailed silvicultural studies in the future.

Chapter 4 Genetic variation

4.1 Introduction

Forest trees are long-lived and immobile organisms and are therefore particularly vulnerable to short-term environmental change. There are increasing concerns about the anthropogenic influence on the forest environment by, for example, tree breeding without regard to genetic diversity (National Research Council 1991), increasing air pollution (Scholz *et al.* 1989) and the perceived threat of global warming (Hattemer and Gregorius 1990, Gravenhorst 1991). The risk of exposure to new diseases is another danger, as illustrated by the American chestnut (*Castanea dentata* (Marshall) Borkh.), which was virtually eliminated by chestnut blight (*Endothia parasitica*), from the eastern United States in the first half of the twentieth century (Schmidt 1978). In the absence of the fungus there had been no selection in the natural population for resistance to it, whereas in China and Japan, where the pathogen occurs naturally, moderate to high levels of resistance are found in chestnut (*Castanea* spp.) populations (Day 1978).

The tree species present in our forest ecosystems today are therefore under considerable anthropogenic influences, both direct and indirect, and if genetic diversity in these species is to be conserved then it is vital that such influences are carefully monitored or controlled. Geographic variation of forest trees was recognised as an important element in breeding programmes from the first half of the nineteenth century (Langlet 1971). Early tree improvement programmes were initially undertaken without any regard for the genetic component of the selected individuals; the main criteria for selection being desirable phenotypic characters, disease resistance or adaptation to the targeted afforestation environment (Morgenstern 1996). Today, the importance of a detailed knowledge of the underlying genetic component of individuals or populations is more widely realised in the forest industry, although research is still guided primarily by a species' economic, rather than ecological, importance (Müller-Starck *et al.* 1992). Tree breeding programmes must address the genetic component as an element in the criteria for selection and indeed, this is increasingly accepted as an important consideration for different species within national tree-breeding programmes (*e.g.* Kitzmiller 1990, Wellendorf 1991, Herzog 1996). In the conservation and management of genetic diversity, the structure of genetic variation must be known in order to make efficient decisions regarding the preservation of genetic diversity, without which, a conservation strategy would involve conserving virtually everything (National Research Council 1991).

Juglans regia is not usually considered endangered but, as a widespread species that is widely cultivated and with disjunct populations, there is likely to have been a significant anthropogenic influence on the temporal integrity of the gene pool. Widespread species have large and complex

genetic structures and concern for the conservation of genetic diversity of such species should therefore be focused on variation at intraspecific levels (Millar and Libby 1991). Widespread species that are economically important therefore require special conservation programmes, since the threat is not to the species' existence but to the integrity of the gene pool (Millar and Libby 1991). For example, in Italy most stands of chestnut (*Castanea sativa*) are affected by disease, enzyme polymorphism is lower in these stands than in Turkish populations (Turkey is thought to be the centre of origin; Villani and Pigliucci 1991), and intraspecific variation is low among grafted orchards in different Italian regions (Fineschi and Malvolti 1991). *Juglans regia* has a spatial distribution and history of cultivation similar to chestnut, which Fineschi and Malvolti (1991) suggested should be considered as a widespread endangered species. The conservation of variation within such widespread species may be considered more meaningful than preserving the last few individuals of a nearly extinct species (Riggs 1990).

Early studies of genetic variation, using quantitative methods to study morphological and physiological traits in woody plant species, found high levels of variation between geographically separated populations (Libby *et al.* 1969). These techniques have been used less frequently to investigate intra-populational variation (*e.g.* Hamrick 1976, Wells and Snyder 1976). Quantitative methods however have inherent shortcomings, namely the inability to separate environmental from genetic effects; phenotypic traits are usually under polygenic control and many important quantitative traits are only expressed after several years (Hamrick *et al.* 1992). The development of genetic markers, particularly protein electrophoresis (isozyme analysis), provided a more direct method to estimate genetic diversity at the species and population levels, and gave access to a large number of simple, codominant markers. Meta-analyses of genetic data combined from studies of woody seed plant species have found a significant correlation between the distribution of genetic diversity and the characteristics of the species' life history and ecology. These studies concluded that long-lived, outcrossing, wind-pollinated and late-successional plants had higher levels of isozyme diversity within populations and less among population diversity than species with other trait combinations (Brown 1979, Hamrick *et al.* 1979, Loveless and Hamrick 1984). A later study by Hamrick *et al.* (1992) supported these findings using seven different traits to classify species: taxonomic status; regional distribution; geographic range; breeding system; seed dispersal mechanism; mode of reproduction; and successional status. *Juglans regia* can be classified according to these traits as a temperate, widespread, mixed-mating and outcrossing, gravity/animal dispersed, sexual and asexual, and late successional angiosperm. These classifications suggest that *J. regia* would have a high diversity, at the species level, and within and between populations. Such categorisation should be undertaken with caution however, as the conclusions of Hamrick *et al.* (1992) were based on gymnosperms (47 % of cases) and

angiosperms (53 %), and on cases based on different sampling strategies (*i.e.* differing number of individuals, populations and loci).

Protein electrophoresis, the most common of which is isozyme analysis, has enabled the determination of genetic diversity in both angiosperms and gymnosperms, since the first published application in forest genetics in 1970 (Feret and Bergmann 1976). Genetic variation studies have since been made on the main forest tree species in Europe (Adams 1983). Studies have now extended to minor tree species, such as *Alnus glutinosa* (Genys 1988) and *Sorbus aucuparia* L. (Raspé and Jacquemart 1998). However, our knowledge of genetic variation within many European tree species remains poor. Müller-Starck *et al.* (1992) revealed that no research had yet been conducted on *Acer, Fraxinus, Tilia* or *Ulmus* by that date.

In the characterisation of genetic variation, isozymes are the most widely adopted and understood of the available marker systems (Butlin and Tregenza 1998). The single most important aspect of the utilised molecular marker in such studies is that it is codominant; *i.e*. both alleles at a locus must be visible in the heterozygous condition. The codominant molecular markers are protein-based (*e.g.* isozymes) and two DNA-based markers; restriction fragment length polymorphism (RFLP) and microsatellites (SSR) (Harris 1999). Dominant markers, such as randomly amplified polymorphic DNAs (RAPD) and amplified fragment length polymorphisms (AFLP), complicate studies of population genetics because the homozygous dominant and heterozygous conditions cannot be distinguished, and their analysis can be further complicated by an unknown number of loci being simultaneously analysed (Gonzalez-Candelas and Palacios 1997).

A summary of the properties and comparative advantages of molecular tools was provided by Karp *et al.* (1997) and Harris (1999). RFLPs are potentially a very useful tool but have a number of shortcomings, in that they are comparatively expensive, very few applicable markers are available, a lot of material is needed to extract DNA (up to 1-1.5 g per extraction) and it is a comparatively slow process (approximately three weeks from tissue extraction to results). Microsatellites (SSRs) are suitable although the generation of primers is expensive, and markers that are generated are usually specific to individual species. With the DNA-based markers, fewer loci may be found although many more alleles per locus are identified compared to isozyme analysis (Karp *et al.* 1997). This high definition makes microsatellite techniques excellent for intra-populational level studies but less useful for interpopulational or species variation analysis (Karp *et al.* 1997). In a comparative study of genetic variation in oak, using microsatellites and isozymes, Degen *et al.* (1999) reported that microsatellite loci had five to six times more alleles than isozyme loci, whilst observed heterozygosity was three times greater in the former compared to the latter. However, genetic distance estimates, although greater for microsatellite data, were

no more precise than for isozyme data. Degen *et al.* (1999) therefore concluded that isozymes are more likely to reveal the impact of different and contrasting population genetic processes. The limitations of isozymes are that only water-soluble enzymes are analysed, the technique is reliant on nucleotide differences leading to changes in charged amino acid composition, and the sampled genes may be a non-representative (small and non-random) sample of the genome (Bergmann 1991).

The study of walnut intraspecific genetic diversity was only one element within this multi-faceted research programme, consequently, there were resource restrictions. Isozymes were the adopted marker system due to the specific properties of the system discussed above and because the development and start-up costs associated with DNA-based markers, both RFLPs and SSRs, for previously unstudied species are greater than with isozymes (Harris 1999). In addition, isozymes permit the analysis of many more samples (up to 10 times more) per day than DNA-based markers, and the level of training required in undertaking isozyme analyses is less than for DNA-based analyses (Karp *et al.* 1997).

Isozyme markers in *Juglans* were first developed in the 1980s. The enzyme systems assayed were AAT and PGI (Arulsekar *et al.* 1985), EST and PGM (Arulsekar *et al.* 1986), and in a comprehensive study of these and an additional nine enzyme systems; ACP, ADH, GDH, LAP, MDH, PRX, SDH, TPI, 6PGD by Arulsekar and Parfitt (1986). Isozymes have been applied in the identification of walnut species or cultivars, to distinguish interspecific hybrids (Arulsekar *et al.* 1985, McGranahan *et al.* 1986, Aleta *et al.* 1990, Germain *et al.* 1993, Solar *et al.* 1993, Solar *et al.* 1994), to characterise walnut mating systems (Rink *et al.* 1989, Malvolti *et al.* 1995), to confirm the mode of inheritance in somatic embryos (Aly *et al.* 1992), and to study genetic variation (Malvolti *et al.* 1993, 1994, 1996).

A limited number of studies of *Juglans regia* intraspecific genetic variation have been conducted. Malvolti *et al.* (1993) studied walnut populations in Italy and reported a F_{ST} value of 0.085, which according to Wright (1978), indicates moderate differentiation among the studied populations. Malvolti *et al.* (1994) assessed genetic variation of central Italian walnut populations by isozyme analysis, in combination with the assessment of quantitative phenological traits. They found average observed heterozygosity was 0.50, which was high compared to a corresponding assessment of 0.31 for *J. nigra* by Rink *et al.* (1989). Only two published studies have investigated intraspecific variation on a broader scale. Within Europe, Malvolti *et al.* (1996) found low levels of genetic variation among western European accessions compared to eastern European accessions, closer to the native natural range of the species. Fornari *et al.* (1999) studied intraspecific genetic variation across both the natural and introduced ranges of *J. regia* but

the populations within the natural range were naturalised rather than indigenous populations, and at two longitudinal extremes (Georgia and China), omitting the substantial central region of the species' natural range. The objectives of the work described in this chapter were therefore to address this lack of knowledge; firstly by undertaking isozyme analyses of the collected genotypes (Chapter 2) and, secondly, to use these data to estimate the genetic structure of the populations.

4.2 Materials and methods

4.2.1 Samples

All walnut plant material, consisting of provenances and land races (as defined in Chapter 2), for which progeny identity had been maintained, were sampled for genetic analysis by isozyme procedures. This consisted of half-sib progenies from 11 Kyrgyz provenances (K1-11), two provenances from Turkey (T1-2) and one provenance from each of Spain (E1), Tajikistan (J1), Iran (P1), Romania (R1), Slovakia (S1), and the UK (U1). For clarity within this chapter, all materials, whether provenances or land races, are referred to as populations.

4.2.2 Isozyme analyses

Comprehensive details of the procedures adopted for the isozyme analyses are given in Appendix IV. These procedures were the result of the laboratory sessions needed to optimise allozyme assay conditions.

Enzymes were extracted from young, healthy leaf samples, collected from one plant of each of the 375 progenies sited at the provenance/progeny field trials (Chapter 5). Other sample material, such as seed embryos and bud meristems, were tested in early laboratory sessions. However, the destructive nature of sampling these tissues excluded their use in the analysis of the genotypes under study because spare seeds or plant material were unavailable. A new extraction buffer, developed for this study, was used in preparing the extracts, which were processed in the field and immediately frozen in liquid nitrogen contained in portable vacuum flasks. The samples were stored at –80 ^{o}C until analysed.

Six enzyme stains produced consistent, resolvable activity and these were used in the final study: aspartate aminotransferase (AAT); aconitase (ACO); isocitrate dehydrogenase (IDH); phosphoglucose isomerase (PGI); phosphoglucose mutase (PGM); and peroxidase (PRX). Nine additional enzymes were resolved in earlier pre-sessions (Appendix IV), using extracts from seed embryos and bud meristems. Electrophoresis was conducted on 13 % starch gels. For ACO,

IDH, PGI, PGM and PRX, histidine citrate gel and electrode buffers (pH 7.5) were used, run at 130 mA/320 V for 6 to 7 hours. Aspartate aminotransferase was run on lithium borate gel and electrode buffers (pH 8.6), at 70 mA/250 V for $3^1/_2$ to 4 hours. Gels were scored when the resolved activity was at its clearest, usually after being stored in 50 % glycerol, at 4 ^oC for approximately 12 hours. A photographic record was maintained of all gels using a 35 mm camera mounted over a light-box.

4.2.3 Data analyses

Data were entered in a Microsoft Excel spreadsheet, which permitted efficient data organisation. Unless otherwise stated, all computations and statistical tests were performed using the population genetics programme Popgene (version 1.31; Yeh *et al.* 1997).

Estimates of genetic variation were calculated for all 19 populations and at the species level. Estimation of observed (H_o) and expected (H_e) heterozygosities (Nei 1975) at a single locus, or over all loci, are a useful and widely adopted measure of genetic diversity (Avise 1994). Observed heterozygosity (H_o) is simply the proportion of all genotypes that were heterozygotes, whilst expected heterozygosity (H_e), also called 'gene diversity' (Nei 1987), is defined as:

$$H_e = \left(1 - \sum_i p_i^2\right)$$

where p_i is the frequency of the i^{th} allele.

Wright's *F*-statistics (Wright 1965) were calculated, including deviations from Hardy-Weinburg equilibrium known as the inbreeding co-efficient (F_{IS}), for each locus in each population. The test statistic (F_{IS}) is the mean deviation from Hardy-Weinburg expectations over all populations and is defined as:

$$F_{IS} = 1 - (H_o / H_e)$$

where H_o is observed heterozygosity, and H_e is expected heterozygosity. As an inbreeding coefficient, F_{IS} provides a measure of heterozygote deficiency or excess, thus if all populations are randomly mating then $F_{IS} = 0$.

The effects of population subdivision, measured by Wright's F_{ST} statistic (Wright 1965), represents the reduction in heterozygosity of a sub-population due to random genetic drift (Hartl and Clark 1989) and is defined as:

$$F_{ST} = \sigma_p^2 \big/ pq$$

where σ_p^2 is the weighted mean of squared deviations of allele frequencies in a population from frequencies over all populations, and *p* and *q* are allele frequencies. The fixation index, F_{IT}, is the correlation among gametes in the total of all populations (Hartl and Clark 1989) and is expressed as:

$$F_{IT} = \overline{F_{IS}} + (1 - \overline{F_{IS}})F_{ST}$$

If all populations are genetically identical then $F_{ST} = 0$ and $F_{IS} = F_{IT}$ while conversely, if all populations are randomly mating then $F_{IS} = 0$ and $F_{ST} = F_{IT}$ (Hartl and Clark 1989).

F_{ST} values were tested for whether they differed significantly from zero using the formula proposed by Workman and Niswander (1970) for cases with codominant alleles:

$$F_{ST'} = F_{ST} - \left(1 \big/ 2N_T \right)$$

where F_{ST} is the estimate of heterogeneity corrected for sampling error, and N_T is the sample size of the total population. The value for F_{ST} is tested against a t-test that assumes that the populations have a F_{ST} value equal to zero.

G-statistics (Nei 1973) were also calculated as these are more appropriate than *F*-statistics for cases, such as this study, when there are more than two alleles at a locus. The average gene diversity within populations (H_S), weighted by population size, is defined as:

$$H_S = 1 - J_S$$

where J_S is the average gene identity within populations (Nei 1973). The average gene diversity between populations (D_{ST}) is defined as:

$$D_{ST} = \sum_k \sum_l D_{kl} \big/ s^2$$

where D_{kl} is the gene diversity between the *k*th and *l*th populations, and *s* is the number of populations (Nei 1973). The gene diversity for the total of all populations (H_T; Nei 1973) was calculated based on the mean allele frequencies over all populations and is expressed as:

$$H_T = H_S + D_{ST}$$

The equivalent measure to F_{ST} is provided by G_{ST} (Nei 1973), which is the measure of the genetic differentiation among populations, relative to the total variation, and is defined as:

$$G_{ST} = D_{ST} \big/ H_T$$

Genetic distance was calculated using the commonly adopted estimate of Nei's standard genetic distance (*D*; Nei 1972), which is corrected when sample sizes are small or unequal by an unbiased estimate (Nei 1978), given by:

$$\hat{D} = -\log_e \hat{I}$$

where $\hat{I} = \hat{J}_{xy} \Big/ \sqrt{\hat{J}_x \hat{J}_y}$ and $\hat{J}$ is the normalised genetic identity $(\hat{J} = 1 - H)$, and *x* and *y* are two populations. Values of *I* can range between zero (when two populations share no alleles) and one (when two populations have identical gene frequencies over all loci; Nei 1972).

D values for protein electrophoretic data are interpreted as the net numbers of codon substitutions per locus that have accumulated since separation of any two populations (Nei 1972). The concept of genetic distance provides a quantitative estimate of the genetic divergence of individuals, populations or taxa (Avise 1994). Measures of genetic distance are used to compare the differences in allele frequencies between all populations using pair-wise comparisons, unlike F_{ST}, which provides a single estimate of the degree of differentiation of populations but no indication of the similarities among individual populations. F_{ST} also relies on populations being in Hardy-Weinburg equilibrium whereas genetic distance (*D*) does not (Hoelzel and Dover 1991). Genetic distances may be summarised using dendograms, one of the simplest of which is the unweighted pair-group method with arithmetic means (UPGMA; Nei 1987). An UPGMA tree was constructed, based on Nei's standard genetic distance, using the GD programme written by Kermit Ritland[1], which estimated genetic distance and its standard error, to produce dendograms with standard errors on the branch lengths (Ritland 1989).

4.3 Results

4.3.1 Isozyme phenotypes

During preliminary studies of young leaf material using six enzyme systems, 19 alleles distributed across 11 loci were identified, five of which were polymorphic (Figure 4.1). During the final laboratory session in which the 375 genotypes were assayed, 15 alleles distributed across 11 loci were identified, two of which were polymorphic.

Aconitase. Two regions of activity were identified during laboratory pre-sessions with young leaf material but these appeared as monomorphic loci in the final analysis of the progenies. Activity in 65 % of the samples (progenies) was successfully resolved.

Aspartate aminotransferase. The best clarity and resolution for this enzyme system was obtained with the lithium borate buffer system. Three regions of activity were initially identified in pre-sessions, although the slowest region (*Aat*-3) was often very faint and sometimes absent. *Aat*-2 had three alleles and was highly polymorphic in the pre-sessions but was monomorphic for all of the sampled genotypes in the final laboratory session. *Aat*-1 remained monomorphic. Sample resolution was 100 %.

[1] http://forgen.forestry.ubc.ca/ritland/programs.html

Isocitrate dehydrogenase. One monomorphic region was consistently present and 75 % of samples were successfully resolved.

Peroxidase. One polymorphic locus, with three alleles, was identified using this system. The fast and intermediate alleles, *Prx*-1*a* and *Prx*-1*b* were closer to each other than to the slow allele, *Prx*-1*c*. Unfortunately, activity was inconsistently resolved in the sample analysis, with only 21 % being successfully resolved.

Phosphoglucose isomerase. Two monomorphic loci were identified and successfully resolved in all samples.

Phosphoglucose mutase. Two regions of activity were identified and successfully resolved in 75 % of the samples. The furthest region of activity from the origin (*Pgm*-1) was polymorphic for three alleles.

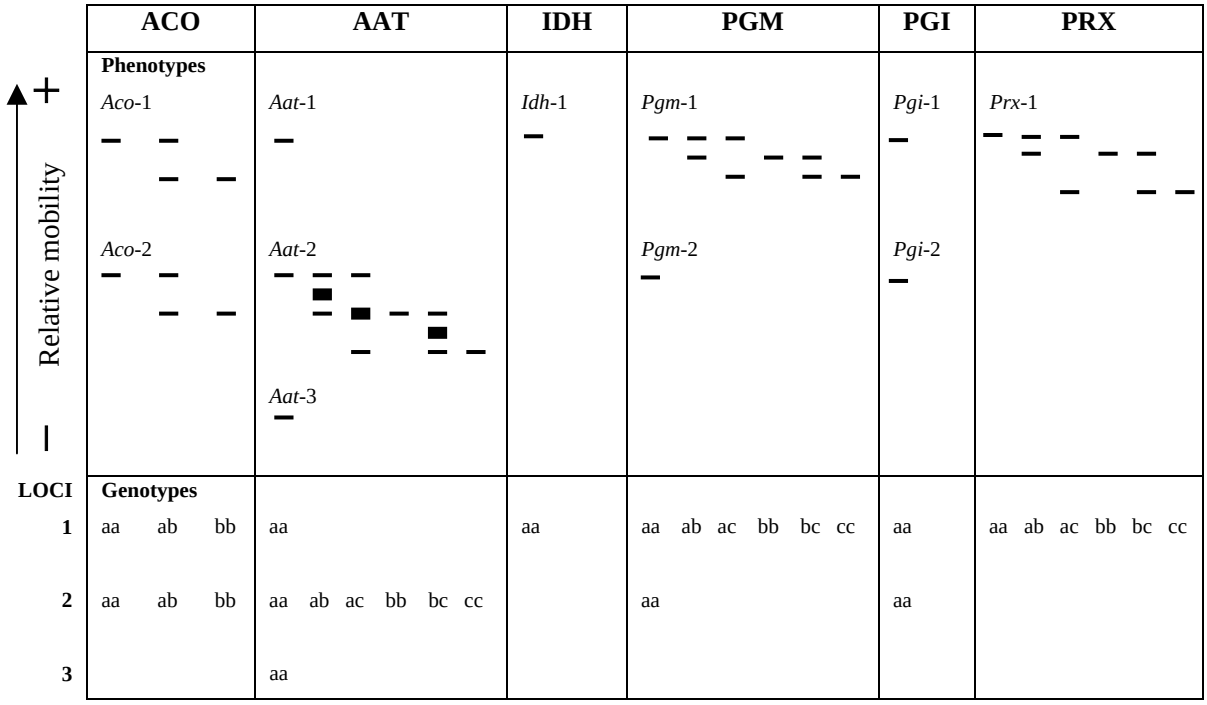


Figure 4.1 Schematic zymograms of enzyme activity detected in *Juglans regia.* Bands of activity are labelled according to their mobility, with the 'faster' (*i.e.* those farthest from the origin) loci being labelled as '1', and 'faster' alleles labelled '*a*'.

The allele frequencies presented in Table 4.1 highlight the low number (21 %) of resolved samples at *Prx*-1. For this reason, data from this locus were not included in any further analyses. Allele frequencies for the only consistent polymorphic locus, *Pgm*-1, were assessed for deviations from Hardy-Weinburg (Table 4.1). Overall, across all populations, there was significant (p=0.001) deviation from Hardy-Weinburg but at the population level only three populations, K3, K11 and R1, significantly deviated ($p \leq 0.050$) from expected allele frequencies. It should also be noted that populations K1, E1, J1, P1 and U1 had very low numbers of samples, consequently the

Hardy-Weinburg calculations for these are subject to large errors, even after correction for small sample size (Levene 1949).

Table 4.1 Allelic frequencies for *Pgm*-1 and *Prx*-1 in 19 populations of *Juglans regia*.

Population	*Pgm*-1					*Prx*-1			
	N	Allele a	Allele b	Allele c	H-W *p* value	*N*	Allele a	Allele b	Allele c
K1	9	0.611	0.056	0.333	0.383	1	0.000	0.500	0.500
K2	15	0.567	0.033	0.400	0.775	2	0.500	0.500	0.000
K3	18	0.611	0.139	0.250	0.002	5	0.400	0.400	0.200
K4	18	0.528	0.139	0.333	0.188	3	0.333	0.667	0.000
K5	13	0.462	0.231	0.308	0.263	5	0.800	0.100	0.100
K6	20	0.650	0.175	0.175	0.664	5	0.600	0.300	0.100
K7	14	0.643	0.107	0.250	0.340	4	1.000	0.000	0.000
K8	17	0.412	0.294	0.294	0.907	3	0.333	0.333	0.333
K9	13	0.539	0.269	0.192	0.204	7	0.429	0.287	0.287
K10	18	0.472	0.139	0.389	0.312	5	0.600	0.400	0.000
K11	22	0.455	0.182	0.364	0.030	9	1.000	0.000	0.000
E1	3	0.667	0.167	0.167	0.954	0	*NA*	*NA*	*NA*
J1	6	0.583	0.250	0.167	0.497	3	0.000	0.667	0.333
P1	3	0.500	0.333	0.167	0.572	0	*NA*	*NA*	*NA*
R1	17	0.529	0.235	0.235	0.006	6	0.333	0.667	0.000
S1	30	0.417	0.367	0.217	0.221	9	0.778	0.167	0.056
T1	18	0.333	0.389	0.278	0.855	6	0.667	0.333	0.000
T2	21	0.500	0.357	0.143	0.850	5	0.600	0.300	0.100
U1	3	0.500	0.500	0.000	0.346	2	0.000	0.500	0.500
Over all Populations	278	0.509	0.227	0.264	0.001	80	0.587	0.306	0.106

(Calculated in Popgene; Yeh *et al.* 1997).

Chi-square tests (χ^2) for Hardy-Weinburg equilibrium were performed on sub-groups of the data, representing regional geographical distributions. There was significant deviation from Hardy-Weinburg for all populations within the natural range (K1-11, J1, P1, T1, T2; p=0.030), and for populations within the introduced range (E1, R1, S1, U1; p=0.010). However, a chi-square test for Hardy-Weinburg equilibrium within the Kyrgyz populations (K1-11) indicated that there was no significant deviation (p=0.060) from Hardy-Weinburg proportions.

There were no cases of rare alleles (frequencies ≤0.005; Hartl & Clark 1989) but this may be due to the low number of samples. In order to detect rare alleles in this study, a more appropriate level associated with a sample size of 10 would be an allele frequency ≤0.01, although there remain no rare alleles at this revised level.

4.3.2 PGM variation within populations

Table 4.2 presents observed and expected heterozygosities at the *Pgm*-1 locus for all populations, and for sub-groups of populations based on their geographical distribution (Chapter 2). The mean genetic diversity estimates for both within populations (H_e) and at the species level was 0.06,

Table 4.2 *Pgm*-1 diversity estimates and inbreeding coefficients in 19 populations of *Juglans regia*. *N*, sample size; H_o , mean observed heterozygosity; H_e , mean expected heterozygosity; F_{IS} , mean inbreeding coefficient; s.e., standard error.

REGION	POPULATION		*N*	H_o	H_e	F_{IS}
Kyrgyzstan (natural range)	K1		18	0.078	0.054	-0.518
		s.e.		0.058	0.040	
	K2		30	0.047	0.054	0.099
		s.e.		0.027	0.031	
	K3		36	0.033	0.056	0.388
		s.e.		0.025	0.030	
	K4		36	0.061	0.061	-0.034
		s.e.		0.032	0.032	
	K5		26	0.085	0.067	-0.324
		s.e.		0.052	0.041	
	K6		40	0.060	0.053	-0.162
		s.e.		0.030	0.026	
	K7		28	0.057	0.053	-0.114
		s.e.		0.034	0.032	
	K8		34	0.071	0.068	-0.074
		s.e.		0.038	0.037	
	K9		26	0.077	0.063	-0.281
		s.e.		0.048	0.039	
	K10		36	0.078	0.062	-0.282
		s.e.		0.041	0.033	
	K11		44	0.091	0.064	-0.447
		s.e.		0.043	0.031	
		Mean	*32*	*0.067*	*0.059*	*-0.159*
		s.e.	2.231	0.005	0.002	0.077
Other natural range populations	J1		6	0.067	0.060	-0.463
		s.e.		0.086	0.077	
	P1		12	0.083	0.062	-0.636
		s.e.		0.076	0.057	
	T1		6	0.100	0.073	0.075
		s.e.		0.129	0.095	
	T2		34	0.094	0.063	-0.028
		s.e.		0.051	0.034	
		Mean	*14*	*0.077*	*0.066*	*-0.263*
		s.e.	6.652	0.009	0.002	0.170
All natural range populations		*Mean*	*27*	*0.069*	*0.061*	*-0.187*
		s.e.	3.083	0.004	0.002	0.070
Introduced range populations	E1		60	0.070	0.066	-0.333
		s.e.		0.029	0.027	
	R1		36	0.061	0.068	-0.545
		s.e.		0.032	0.036	
	S1		42	0.062	0.062	-0.085
		s.e.		0.030	0.030	
	U1		6	0.033	0.060	0.333
		s.e.		0.043	0.077	
		Mean	*36*	*0.066*	*0.062*	*-0.158*
		s.e.	11.225	0.012	0.001	0.189
All populations		*Mean*	*29*	*0.069*	*0.061*	*-0.181*
		s.e.	3.306	0.043	0.001	0.066
Species level			556	0.068	0.062	-0.103

whilst between populations H_e ranged between 0.05 and 0.07 (Table 4.2). Comparisons between H_o and H_e (Table 4.2) indicate that only four (K2, K3, T1, U1) of the 19 populations had H_e values greater than H_o, although the large standard errors indicate that these differences were non-significant. To explore further any relationship between genetic diversity and geographical distribution, H_o and H_e were plotted against latitude and longitude of origin (Figure 4.2) but these indicated that there were no relationships between these parameters.

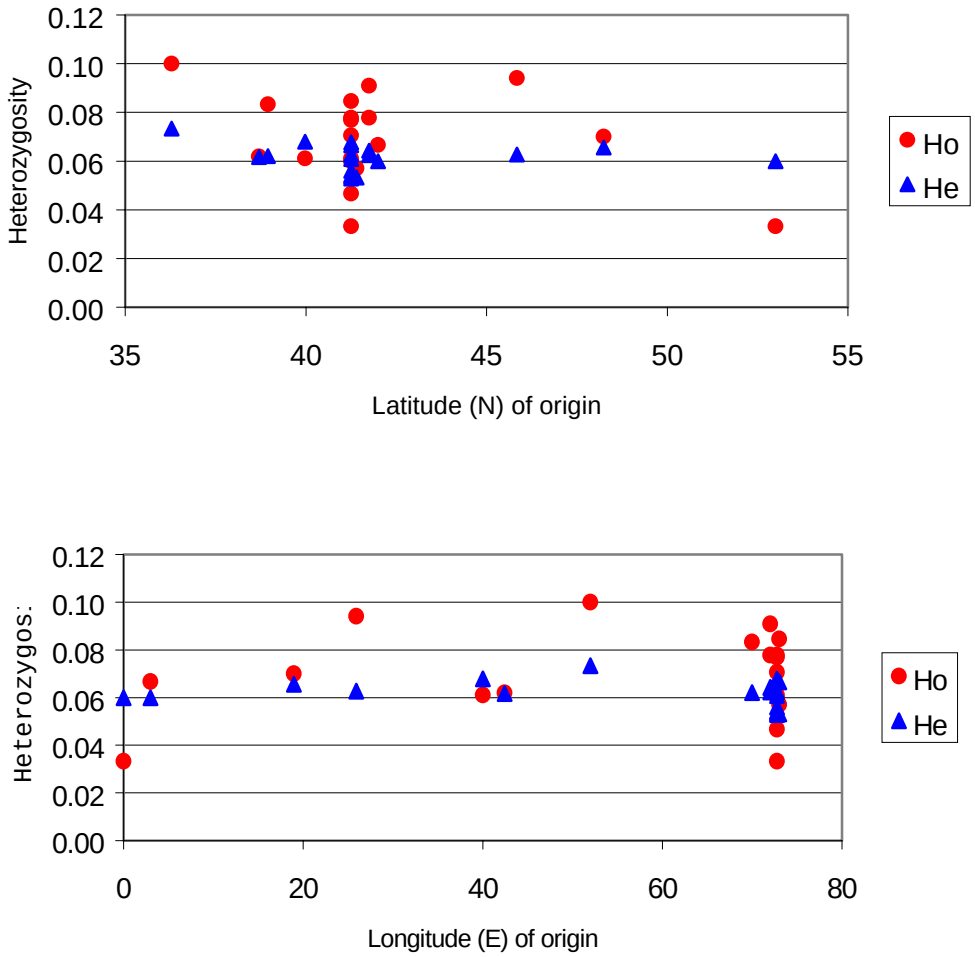


Figure 4.2 Observed (H_o) and expected (H_e) heterozygosities of 19 populations of *Juglans regia*, plotted against latitude and longitude of origin.

The inbreeding coefficient for genotypic proportions indicated that over all populations, F_{IS} was negative, signifying that more heterozygotes were present in the sampled populations than would be expected under random mating. However, sample sizes may not have been large enough to detect homozygotes since under Hardy-Weinburg equilibrium, twice as many heterozygotes would be expected in any sample (genotype frequencies for one locus with two alleles would be p^2, $2pq$, and q^2 for genotypes *AA*, *Aa* and *aa*). Analysis of 38 χ^2 comparisons for deviations from

Hardy-Weinburg (F_{IS}) showed that only three cases (16 %) were significant at $p<0.050$: populations K3, K11 and R1, the latter two of which had an excess of heterozygotes (Table 4.2).

The mean total gene diversity (H_T) was 0.611 whilst mean gene diversity among populations (D_{ST} = 0.031; H_S = 0.580) indicated that most of the genetic diversity occurred within populations.

4.3.3 PGM variation between populations

F_{ST} values presented in Table 4.3 indicate that at the species level, only 5 % of the total *Pgm*-1 variation was accounted by differences among populations, the remainder was found within populations. F_{ST} values for the two natural range groups indicated that <3 % of the variation was distributed between populations within these groupings. F_{ST} values corrected for sampling error (Table 4.3) indicate that over all populations, and within regional groups, all estimates were significantly different from zero.

Table 4.3 Summary of *F*-statistics (Wright 1965) for populations among regions at the *Pgm*-1 locus.

Regional group	*N*	F_{IS}	F_{IT}	F_{ST}	$F_{ST'}$ *
Kyrgyzstan populations (K1-11)	177	-0.159	-0.131	0.029	0.027 0.051
Other natural range populations (J1,P1,T1,T2)	48	-0.263	-0.227	0.022	0.012 0.029
All natural range populations	225	-0.187	-0.140	0.042	0.039 0.022
Introduced range populations (E1, R1,S1,U1)	53	-0.158	-0.107	0.055	0.046 0.042
Species level	278	-0.103	-0.125	0.051	0.049 0.055

* $F_{ST'}$ is the estimate of heterogeneity corrected for sampling error (Workman and Niswander 1970). Figures in small type indicate approximate sampling errors.

The overall degree of genetic differentiation between populations (G_{ST}) was 0.050, indicating that approximately 95 % of genetic variation at the *Pgm*-1 locus resided within populations.

4.3.4 Population differentiation

Table 4.4 presents values of unbiased genetic distance ($\hat{D}$) for all pair-wise comparisons of populations, whilst a summary of genetic distance values for regional groups is given in Table 4.5. *D* values range from zero to infinity, and where these values are negative one may assume that $D = 0$ (Nei 1987).

Table 4.4 Nei's unbiased measures of genetic distance (Nei 1978) for 19 populations of *Juglans regia* at the *Pgm*-1 locus.

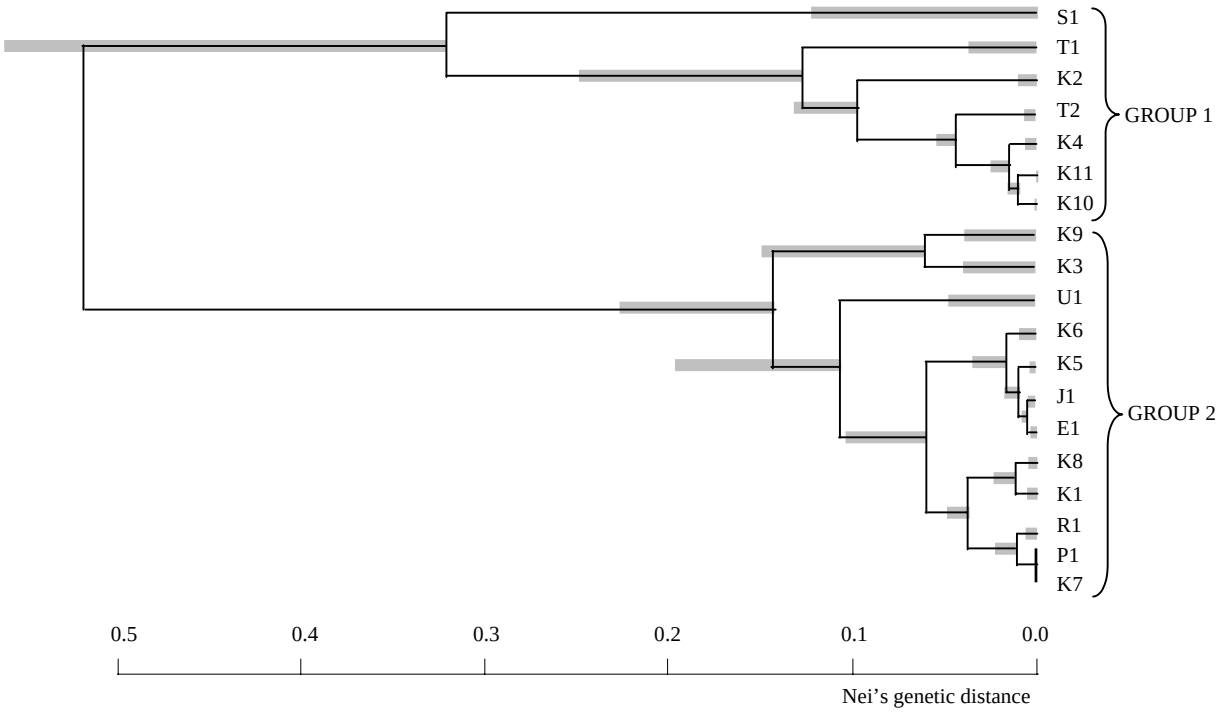
Pop.	K1	K2	K3	K4	K5	K6	K7	K8	K9	K10	K11	E1	J1	P1	R1	S1	T1	T2	U1
K1	****	-0.0009	-0.0004	-0.0005	0.0014	0.0010	-0.0007	0.0039	0.0023	0.0003	0.0010	-0.0009	0.0014	0.0025	0.0013	0.0068	0.0089	0.0062	0.0139
K2		****	0.0010	0.0000	0.0020	0.0033	0.0008	0.0045	0.0042	0.0001	0.0010	0.0015	0.0036	0.0045	0.0027	0.0081	0.0095	0.0085	0.0175
K3			****	-0.0001	0.0007	-0.0003	-0.0008	0.0026	0.0003	0.0012	0.0013	-0.0022	-0.0007	-0.0003	0.0000	0.0041	0.0066	0.0030	0.0081
K4				****	-0.0004	0.0014	0.0002	0.0011	0.0009	-0.0006	-0.0004	-0.0003	0.0005	0.0001	0.0001	0.0034	0.0046	0.0037	0.0100
K5					****	0.0020	0.0016	-0.0008	-0.0002	-0.0003	-0.0008	0.0004	-0.0001	-0.0019	-0.0006	0.0006	0.0011	0.0013	0.0059
K6						****	-0.0003	0.0036	0.0002	0.0034	0.0032	-0.0027	-0.0011	-0.0008	0.0003	0.0043	0.0075	0.0023	0.0057
K7							****	0.0038	0.0010	0.0017	0.0020	-0.0023	-0.0002	0.0007	0.0006	0.0056	0.0084	0.0041	0.0097
K8								****	0.0003	0.0010	0.0001	0.0022	0.0007	-0.0021	0.0001	-0.0002	-0.0002	0.0009	0.0043
K9									****	0.0021	0.0013	-0.0016	-0.0018	-0.0033	-0.0009	0.0004	0.0023	-0.0004	0.0018
K10										****	-0.0007	0.0018	0.0022	0.0013	0.0010	0.0038	0.0041	0.0050	0.0122
K11											****	0.0017	0.0015	0.0001	0.0005	0.0024	0.0026	0.0036	0.0098
E1												****	-0.0030	-0.0024	-0.0014	0.0029	0.0063	0.0006	0.0040
J1													****	-0.0035	-0.0014	0.0007	0.0032	-0.0007	0.0014
P1														****	-0.0026	-0.0027	-0.0012	-0.0033	-0.0024
R1															****	0.0008	0.0024	0.0004	0.0038
S1																****	-0.0002	0.0000	0.0011
T1																	****	0.0016	0.0033
T2																		****	-0.0006

Two main clusters or groups are evident in the dendrogram (Figure 4.3a) and the mean values of $\hat{D}$ for these two groups are presented in Table 4.5. The node of Group 1 has a large standard error, as indicated by the long error bar length, signifying that there is no reason to suspect that this grouping is any different than would be expected from a random grouping. Population S1 is significantly different from other populations within Group 1. Group 2 is a well defined group with a low standard error, as indicated by the relative short error bar length. This grouping does not reflect the geographic origin of the populations as it contains those from both the natural and introduced ranges.

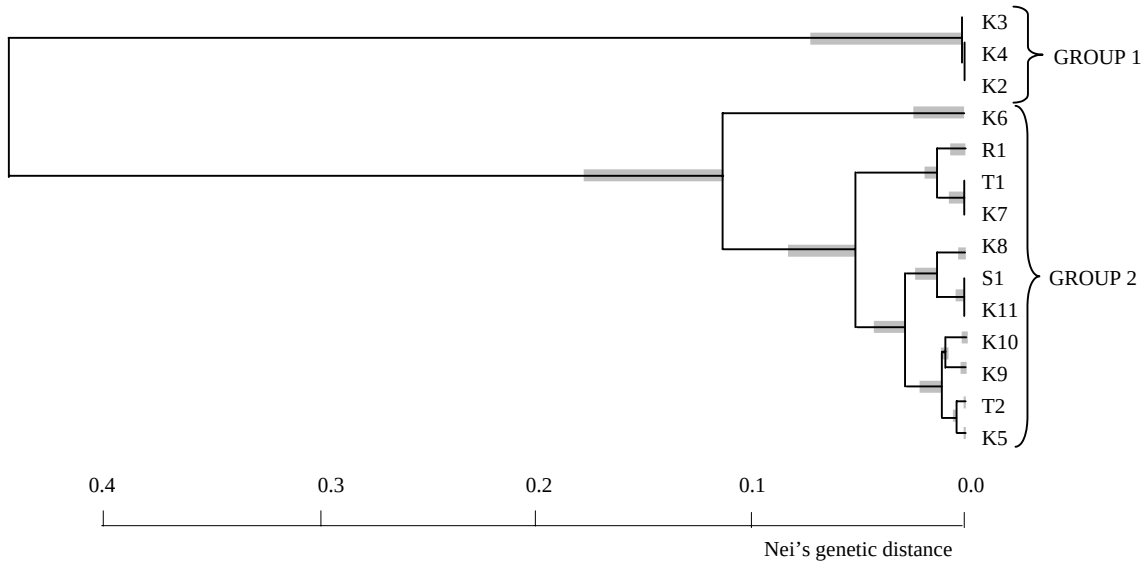
Table 4.5 Unbiased genetic distance ($\hat{D}$) (Nei 1978) estimates for population regional groups, and for groups and sub-groups within dendrogram (Figure 4.3).

Group/Sub-group	*N*	mean	s.e.
Kyrgyz populations	55	0.001	0.000
Fergana group (K1-9)	36	0.001	0.000
Chatkal group (K10-11)	1	*0.000	*NA*
Other natural range populations	6	*0.000	0.001
All natural range populations	105	0.001	0.000
Introduced range populations	6	0.002	0.001
All populations	*171*	*0.307*	*0.013*
Dendrogram group 1	21	0.003	0.001
Dendrogram group 2	66	0.001	0.000

* negative values transformed to $\hat{D}$ = 0



(a)



(b)

Figure 4.3 UPGMA cluster analysis of *Juglans regia* populations based on Nei's standard genetic distance (Nei 1972) for: (a) all 19 populations, and (b) 14 populations with sample size ≥ 13. Broad grey lines indicate one standard error of genetic distance, and where these are less than 50 % of the branch length, they indicate a significant cluster (Ritland 1989). A key to population abbreviations is given in Appendix I.

The effect of sample size on the cluster analysis was explored by progressively removing populations with low sample numbers from the analysis. Group 2 (Figure 4.3a) remained discrete, even when only populations with population sizes greater than 19 were analysed. However, when populations with fewer than 13 samples were excluded from the analysis (K1, E1, J1, P1 and U1), two significant groups were identified (Figure 4.3b). Populations K2, K3 and K4 (Group 1; Figure 4.3b) formed a distinct group, although these did not correlate with geographical origins, such as shared watersheds (Figure 2.2, p.17). The inclusion of populations from the introduced range (R1 and S1) with those from the natural range within Group 2 (Figure 4.3b), indicates that these introduced populations are likely to have originated from within this group, which includes the two Turkish populations sited on the border of the natural and introduced ranges.

4.4 Discussion and conclusions

Preliminary studies identified 20 loci in 15 enzyme systems using seed embryo extracts (Appendix IV) but these results could not be replicated with extracts from young leaf material. The necessity of using leaf extracts in this study resulted in poorer enzyme resolutions, and the generation of less data for use in genetic diversity studies than if seed embryo extracts had been used. Solar *et al.* (1994) reported better resolution with pollen extracts than with young leaves. The failure in resolving any polymorphic activity in ACO and AAT during final laboratory sessions, when the genotypes were assayed, cannot easily be explained. Enzyme activities can be influenced by differences in the physiological and ontogenetic condition of the tissue samples or even changes in the environment during sampling (Wendel and Weeden 1990). In this study, preliminary samples were collected from young seedlings under the cover of a polythene tunnel, and from the young leaves of mature trees, whilst the genotypes were sampled exclusively by extracting tissues from young leaves of one-year-old seedlings in open-growing conditions.

Previous protein electrophoretic studies of *Juglans regia* have identified only five polymorphic systems: EST and PGM (Arulsekar *et al.* 1986) and MDH, SDH and 6-PGD (Aleta *et al.* 1990, Solar *et al.* 1994). The existence of two zones of PGM activity in this study, *Pgm*-1 being polymorphic with three alleles and *Pgm*-2 being monomorphic, is in agreement with the results described by Aleta *et al.* (1990) and Arulsekar *et al.* (1986). The other four systems, where polymorphic activity has been identified using leaf extracts (EST, MDH, SDH, 6-PGD; Aleta *et al.* 1990, Solar *et al.* 1994, Arulsekar *et al.* 1986), were not successfully resolved in the progeny study.

Two previously untested enzyme systems were successfully resolved in this study; one monomorphic zone of IDH activity was resolved, whilst in preliminary sessions using leaf extracts, ACO was found to have two polymorphic loci, each with two alleles. Of the three AAT loci, *Aat*-2 was polymorphic with three alleles in this study where previously, three (Aleta *et al.* 1990, Arulsekar *et al.* 1986) or four (Solar *et al.* 1994) poorly resolved zones of activity had been reported. The poor separation of PRX alleles from leaf extracts was also described by Solar *et al.* (1994), although three polymorphic loci were identified by these authors.

Long-lived, woody perennials generally maintain higher levels of genetic diversity within populations and at the species level, compared to other organisms (Hamrick and Godt 1989, Hamrick *et al.* 1992). In this study, the assessment of data from a single polymorphic locus precluded the adoption of two commonly adopted indicators of genetic diversity: the mean number of alleles per locus (*A*) and the proportion of polymorphic loci (*P*). However, there is an argument that these are not good measures of diversity as *A* is subject to the problems of sample size variation unless some process of rarefaction is used on the data, whilst *P* is dependent on the magnitude of the exclusion criterion. Such considerations have not prevented the promotion of *A* as the most important criterion for the estimation of diversity, indeed the estimation that a sample size of between 20-30 individuals per population is sufficient (Brown and Marshall 1995) is based on these parameters. Expected heterozygosity (H_e) estimates for *Juglans regia* calculated in this study, both within populations (0.06) and at the species level (0.06), are exceeded by mean values for H_e reported by Hamrick *et al.* (1992) for long-lived woody perennials, 0.148 and 0.177 respectively. Genetic diversity estimates (H_e) for European broadleaved tree species in more recent studies also exceed the values estimated here for *J. regia*: *Quercus petraea* and *Q. robur* (0.245 and 0.252 respectively; Zanetto *et al.* 1994), *Sorbus aucuparia* (0.229; Raspé and Jacquemart 1998). However, these comparisons are not justified since the data presented here is based on a single polymorphic locus, for which the maximum theoretical diversity occurs when $p=q=r=0.33$, *i.e.* $H_e = 0.67$.

Single-locus estimates for H_e are infrequently specified in publications. Zanetto *et al.* (1994) provided H_e values at *Pgm*-1 for *Quercus robur* and *Q. petraea* of 0.502 and 0.150 respectively, although the mean number of alleles (*A*) were greater for these species (3.714 and 2.857 respectively) than for walnut. In a study of only 26 open-pollinated *Juglans nigra* families, Rink *et al.*(1989) estimated a H_e value of 0.315 at the *Pgm*-1 locus, which was greater than the mean H_e value of 0.264 over eight loci. Malvolti *et al.* (1993) analysed 11 Italian populations of *J. regia* using 294 samples, based on 10 enzyme systems [AAT, ADH, IDH, aryl alcohol dehydrogenase (AADH; E.C. 1.1.1.90.), diaphorase (DIA; E.C. 1.6.99.-), superoxide dismutase (SOD; E.C. 1.15.1.1.), PGM, EST, mannose phosphate isomerase (MPI; E.C. 5.3.1.8.), PGI] and 16 loci.

Malvolti *et al.* (1996) studied populations from the Caucasus, France, Greece, Hungary, Italy and Spain, using both isozymes and DNA markers. Malvolti *et al.* (1993) calculated a mean H_e value for *J. regia* of 0.142 (population H_e values ranged between 0.118 and 0.159), although no single locus estimates of H_e were provided. Malvolti *et al.* (1996), in which enzyme system details were not included, estimated a mean H_e value across all populations of 0.191 (s.e. 0.007). The low estimates of genetic diversity for *J. regia* in this study, indicated by low H_e values, may be due to several factors, particularly the use of data from a single locus with three alleles. Hartl *et al.* (1994) showed that estimates of *H*, for sample sizes between 30 and 400, were significantly negatively correlated with the number of loci investigated. Some of the more readily assayed enzymes and hence most widely used systems, such as the esterases, peptidases, and some oxidoreductases and isomerases, are the most variable systems (Vallejos 1983, Hartl *et al.* 1994). For example, with oak (*Quercus* spp.), Zanetto *et al.* (1994) found that enzymes involved in the primary metabolism (Group I; Bergmann 1991) exhibited higher heterozygosities than enzymes involved in the secondary metabolism (Group II; Bergmann 1991). The proportion of enzymes adopted within a study, in respect to their involvement in different metabolism groups, may therefore influence the results.

Hamrick *et al.* (1979) reported that many conifer species had F_{ST} values of <0.1 indicating a high degree of uniformity among populations; *i.e.* less than 10 % of the total genetic variation is accounted for by differences among populations, the remaining 90 % is contained within populations. Among temperate angiosperms, the overall level of differentiation, as measured by F_{ST} or G_{ST} values, varies between species. G_{ST} values reported for *Quercus petraea* range between 0.025 (Zanetto and Kremer 1995) and 0.032 (Zanetto *et al.* 1994), *Q. robur* (0.024; Zanetto *et al.* 1994), *Fagus sylvatica* values ranged between 0.014 and 0.040 (Hazler *et al.* 1997), and *Sorbus aucuparia* (0.060; Raspé and Jacquemart 1998). Within Italian populations of *Juglans regia*, Malvolti *et al.* (1993) reported a F_{ST} value of 0.085, indicating that 91.5 % of the species' total variation was distributed across populations. In the same study at the *Pgm*-1 locus only, Malvolti *et al.* (1993) estimated a F_{ST} value of 0.100, although no error is given. Malvolti *et al.* (1996) calculated G_{ST} values (Nei 1973), which ranged between 0.025 and 0.041 for populations from Italy, 0.047 in Sicilian populations, 0.021 (lowest) in French populations, and 0.132 (highest) for Caucasian populations. G_{ST} estimates by Fornari *et al.* (1999) indicated greater diversity for Chinese and Caucasian populations (0.106) than European populations (0.066). The species level F_{ST} and G_{ST} values, both of which were 0.05 in this study (Table 4.3), indicate very high uniformity among populations. Among regional groups, F_{ST} values were greatest among the four populations within the species introduced range (0.055) and least for non-Kyrgyz natural range populations (0.022). The low F_{ST} values reported here are to be expected given the similarity of allele frequencies between populations (Table 4.1, p. 79). If one allele is consistently rare across

populations, then high F_{ST}/G_{ST} values will result. Indeed, the allele frequencies provided by Malvolti *et al.* (1993) indicate a lower frequency for allele *a* at *Pgm*-1, which may explain their higher F_{ST} estimate.

Genetic distance estimates of the *Juglans regia* genotypes, based on the *Pgm*-1 locus, did not highlight any significant clustering consistent with the geographic distribution of sample origins. Given the allele frequencies reported in Table 4.1 and the use of data from a single locus, these results are predictable. Nei (1978) recommended that a large number of loci should be used to obtain a reliable estimate of genetic distance, particularly when genetic distance is small. When those populations with low (<13) numbers of samples were excluded from the analysis, two distinct groups were indicated, signifying that some populations within Kyrgyzstan were more similar to populations further west within the natural range (Turkey) and in the introduced range (Romania and Slovakia). Malvolti *et al.* (1996) concluded that the lower degree of genetic diversity found in western populations of *J. regia*, compared to eastern populations close to the natural range, reflected strong human pressure of the gene pool.

The results of this study, comparing a wider collection of material from both within the natural and the introduced ranges of *Juglans regia*, do not support the findings of Malvolti *et al.* (1996). This may be explained by the use of data obtained from a single locus in this study. The closest study providing a comparison of PGM variation, of 11 Italian populations by Malvolti *et al.* (1993), estimated an F_{ST} value twice that calculated in this study but these authors did not indicate an associated error. Given the large error associated with the F_{ST} estimate given here, there is no evidence that these two estimates differ significantly. The more recent paper by Fornari *et al.* (1999), in which material from a wider range was tested, relied on the work of separate laboratories. Their findings, that one allele at *Pgm*-1 was absent from Chinese populations, cannot be relied upon without verification that controls were used to standardise the results across the different laboratories. The populations sampled within this research programme not only represented the introduced range, where anthropogenic influence may have been significant, but also those populations from within the natural range where it was thought that the maximum amount of genetic diversity may have been retained. The results of this study, indicating low estimates of genetic distance and high uniformity both within and between populations, indicate that *J. regia* should be considered an endangered widespread species. Further measures to identify and conserve existing genetic variation should therefore be undertaken.

Chapter 5 Provenance/progeny trials

5.1 Introduction

The stages necessary for the successful implementation of a tree introduction or breeding programme are numerous and involve the combination of many factors (Williams and Matheson 1994). The first stages in the walnut programme have been described, namely the definition of the programme objectives (Chapter 1), the collecting strategy (Chapter 2) and the raising of seedlings for inclusion in field trials (Chapter 3). This chapter describes the next stages in the research programme, namely the definition of the field trials' objectives, description of the material tested and details of the trial design. Finally, data resulting from assessments made during the first year of growth in the field trials are presented.

AIMS AND OBJECTIVES

The aims of the provenance/progeny trials are to test the performance of the sampled genotypes in environmental conditions typical of suitable forestry sites in southern England, and to assess their suitability for producing quality hardwood timber. Most of this genetic material is being tested in Britain for the first time and these trials provide a unique opportunity for the long-term assessment of the species' future potential as an economic crop. This project has laid the foundations for a long-term research programme and many of the main aims and objectives lie beyond the scope of this thesis (Figure 5.1).

D.Phil. research project:

- To measure early growth and to record survival of the collected genotypes and to analyse these factors in relation to provenance origin and parent tree characteristics.
- To study some parameters of phenological variation and to assess these both in relation to environmental variables of origin and to intraspecific genetic variation revealed by isozyme analyses.
- To evaluate genotype × environment interaction, as provided by the three field trials planted in the southern England.

Long term:

- To identify provenance locations where further sampling would be desirable.
- To quantify the heritabilities of desirable traits (such as form, growth rate and timber quality) in the genotypes.
- To measure growth rates, in terms of volume production (height and *dbh*) with age, to facilitate the production of yield tables.
- To select genotypes suitable to include in breeding programmes, leading to the availability of improved walnut for the forestry industry.

Figure 5.1 Objectives of the provenance/progeny field trials.

MATERIAL

The walnut genetic material collected across both the natural and introduced ranges of *Juglans regia* (Chapter 2) and propagated during 1998 (Section 3.1), was planted-out in three field trials sited in southern England during December 1998. Twenty-five provenances and 375 half-sib progenies are distributed across three provenance trials located on different sites, the largest of which also acts as a combined provenance/progeny trial. A proportion of the material included in the field trials is not strictly a 'provenance', as defined in Chapter 2 but would be more appropriately referred to as a 'land race' of unknown original source. However, for simplicity within this chapter, all material is referred to as a provenance or progeny, with some clarification of definition where appropriate.

5.2 Trial design

Cox (1958) defined five main requirements of good experimental design. They are the absence of systematic error, the need for precision, range of validity, simplicity and calculation of uncertainty. These rules were followed in the design of the walnut field trials with the ultimate aim of separating differences between treatments (provenances and progenies) from uncontrolled (*e.g.* environmental) variation that is assumed to be present. The trial designs were also formulated with regard to the intended methods of analysis, as recommended by Mead (1988).

The design of the field trials was complex for a number of reasons. The value of the material included in the trials is great, both in respect to being an unevaluated genetic resource and as potential stock for future breeding programmes. The trials would be required to act as a sampling bank of material for genetic analysis within this project (Chapter 4) and in addition, much of this material had not previously been tested in Britain. As much material as possible therefore needed to be included in the trials. In many cases however, there were low numbers of plants for individual progenies and provenances leading to imbalance within and between provenances. The resulting lack of orthogonality was the only desirable feature of experimental design (Cox 1958) which was not fulfilled in the design of the field trials. However, it may be argued that the influence of modern computers and statistical methods has subsequently reduced this requirement (Mead 1988).

For reasons of clarity, the designs of the provenance trials and the progeny trial, which is combined within one of the provenance trials, are described separately below.

5.2.1 Provenance trials

All plant material successfully propagated during 1998 was incorporated in the provenance trials, resulting in the inclusion of 25 provenances (Table 5.1). To allow statistical analyses to be undertaken, the original coding system for the provenances/progenies was converted to a continuous numerical form. The provenances are simply numbered from 1 to 25 (*e.g.* K1 = 1, K11 = 11, E1 = 12) (Table 5.1) (A fold-out summary is provided in the appendix (Table I.1)). Similarly, progenies are sequentially numbered from 1 to 375 and therefore lose their immediate reference to provenance identity (*e.g.* progeny 61 was labelled K4.7, thereby indicating its nesting within Kyrgyz provenance 4). Both coding systems are used in the text where possible but statistical analyses were limited to numerical codes.

The provenance field trials were planned as a randomised complete block (RCB) design incorporating non-contiguous multiple-tree plots distributed in twenty two 100-tree blocks, located across three sites. The largest trial, sited in Oxfordshire contains 14 blocks of the 22 (1400 trees), whilst the other two trial sites each contain four blocks (400 trees). At sites one (Oxfordshire) and two (Gloucestershire), the blocks are square in design (10 × 10 trees) whilst on site 3 (Somerset), the dimensions of the area necessitated a rectangular block design. Each 100-tree block is split into four 25-tree sub-blocks, thereby making the trial design more robust by providing some control over within block variability, which could be significant, given the area of the blocks (2025 m^2) (Cox[1], pers. comm.). The sub-blocking also provided a method by which to stratify the random distribution of the provenances within a block; *i.e.* so that each provenance is distributed across the whole block, thereby preventing a clustered distribution. The trial plans presented in Figure 5.2 (p. 93), show the distribution of one provenance and its progenies, providing an example of the design.

Three trial sites were selected for planting to permit the assessment of genotype × environment interaction (GEI). They were selected according to their suitability for walnut cultivation (Section 3.2.1) and for their contrasting environmental conditions (Table 5.2, p. 94). Soil samples were taken from each site at planting time, consisting of a bulked sample collected from 25 randomly located sample points distributed across the planting site, to a depth of 20 cm using a soil auger.

[1] Professor Sir David Cox, FBA, FRS, Department of Statistics, University of Oxford.

Table 5.1 Summary of provenances incorporated in field trials

Provenance number	Provenance code	Provenance name	Country of origin	Latitude (°N)	Longitude (°E)	Altitude (m)	Original material [1]	Number of progenies	Number of trees in trials
1	K1	Ak-Terek	Kyrgyzstan	41.25	72.75	1700	Progeny seed	11	23
2	K2	Ak-Terek	Kyrgyzstan	41.25	72.75	1390	Progeny seed	17	95
3	K3	Ak-Terek	Kyrgyzstan	41.25	72.75	1860	Progeny seed	26	152
4	K4	Sharap	Kyrgyzstan	41.25	72.75	1620	Progeny seed	26	161
5	K5	Yaradar	Kyrgyzstan	41.25	73.00	1260	Progeny seed	20	140
6	K6	Shaidan	Kyrgyzstan	41.25	72.75	1590	Progeny seed	26	184
7	K7	Kyzyl-Ungur	Kyrgyzstan	41.40	73.00	1400	Progeny seed	17	151
8	K8	Katar-Yangak	Kyrgyzstan	41.25	72.75	1900	Progeny seed	24	167
9	K9	Kyok-Sarau	Kyrgyzstan	41.25	72.75	1830	Progeny seed	26	190
10	K10	Kyr-Sai	Kyrgyzstan	41.75	72.00	1320	Progeny seed	27	208
11	K11	Ters-Kolt	Kyrgyzstan	41.75	72.00	1440	Progeny seed	27	191
12	E1	Catalonia	Spain	42.00	3.00	175	Progeny seed	5	44
13	J1	Tadjikistan	Tadjikistan	38.95	70.00	*	OP cultivar	7	60
14	P1	Karaj	Iran	36.28	52.00	*	OP cultivar	5	38
15	R1	Romania	Romania	45.84	25.90	478	Progeny seed	27	63
16	S1	Slovakia	Slovakia	48.24	18.96	215	Progeny seed	34	107
17	T1	Trabzon	Turkey	39.98	40.00	776	Progeny seed	25	50
18	T2	Anatolia	Turkey	38.70	42.45	1650	Progeny seed	21	38
19	B1	Rossosh	Russia	50.25	39.50	95	Bulked provenance seed	*	10
20	B2	Kourpat	Ukraine	48.00	25.50	350	Bulked provenance seed	*	8
21	A1	Cauc 26	Georgia	43.00	45.00	*	OP cultivar [t]	*	40
22	F1	RA464 Lozerrone	France	48.00	0.00	*	OP cultivar	*	24
23	F2	RA611	France	48.00	0.00	*	OP cultivar	*	15
24	F3	Charentes	France	48.00	0.00	*	OP cultivar [t]	*	24
25	U1	UK	United Kingdom	53.00	0.00	*	Progeny seed	4	16
							TOTAL	**375**	**2199**

[1] 'OP cultivar' is a collection from open-pollinated cultivars and those marked ([t]) were supplied as transplants (not seed).

Table 5.2 Walnut provenance/progeny trials – summary of site and trial properties.

		Paradise Wood, Oxfordshire	**Northwick Estate, Gloucestershire**	**Maunsel Estate, Somerset**
Map reference (O.S. 1:50,000)		SU 554 938	SP 146 366	ST 305 298
Latitude / Longitude		51 º 38′ N / 1 º12′ W	52 º 2′ N / 1 º 47′ W	51 º 3′ N / 3 º 0′ W
Altitude (metres above sea level)		50	245	15
Aspect (º)		Flat	145	0
Slope		Flat	Hill top/ gentle slope	Gentle slope
Soil	pH	7.6	8.0	7.3
	N (mg/l)	4	14	14
	P (mg/l)	19	16	17
	K (mg/l)	153	107	120
	Mg (mg/l)	115	25	78
	texture	Sandy clay loam	Sandy silt loam	Sandy clay loam
Geology [1]		Valley gravel	Inferior Oolite	Upper (Keuper) Marls
Previous use of site		Arable	Arable	Grass pasture (at least 10 years)
Site preparation		Ploughed/cultivated/sown with grass	Ploughed/cultivated	None
Number of trees		1400	400	400
Spacing (metres)		5 x 5	4 x 4	3.8 x 3.8
Protection		0.75 m tubes	0.75 m tubes	0.75 m tubes
Data level		Provenance and progeny	Provenance only	Provenance only

[1] Ordnance Survey of England (1907, 1929, 1948).

The samples were analysed[2] for pH, main chemical constituents and texture (Table 5.2). The soil pH on all sites is close to the recommended range for walnut silviculture, pH 6.5 – 7.5 (Becquey 1997) or pH 6 - 7 (Evans 1984), although a wider range of pH 5.5 –8.5 is specified by Klemp (1979). The sites differ widely in topographical conditions (Plates 5.1, 5.2 and 5.3) and should provide contrasting test conditions.

The variation between the number of replicates, both within and between provenances, and between sites, has created an imbalance in the trials (Table 5.3, p. 98). Variation in replicate numbers within each provenance has been limited, where possible, to ± one replication within each site (Table 5.3). Eighteen provenances are replicated across all blocks and all three trial sites, whilst the seven remaining provenances, which are bulked provenances, are present only on the two smaller sites (Table 5.3). The bulked provenances consist of very low numbers of trees and have not been considered at great length here, although they provide some indication of contrasting phenotypic variation from the provenance material.

Within a block each tree has a numbered position and these flow in alternate directions to minimise walking distance whilst making assessments, and they follow the direction of herbicide treatments to minimise ground disturbance.

The trees are spaced at the widest practicable spacing on each site. A suitable spacing was calculated to be 5 × 5 m, based on the thinning regime based on a crown and stem diameter regression (Section 3.3.4). At this spacing, crown canopy cover can be estimated to be at 100 % when *dbh* reaches 15 cm which, based on an approximate annual stem increment of 1 cm year $^{-1}$ (Evans 1984, Becquey 1997), could be attained in 15 to 20 years. The trial could therefore continue without intervention for this period, thereby allowing sufficient time for the proper assessment of the genotypes before thinning of some trees may be necessary. The Oxfordshire trial was planted at 5 × 5 m whilst the smaller areas available at the other sites necessitated a reduction in spacing to 4 × 4 m in Gloucestershire, and 3.8 × 3.8 m in Somerset. On each site the trial material in the blocks is surrounded by one ‘guard’ row on all

[2] Analysis was undertaken by A.D.A.S. Consulting Limited, Woodthorne, Wergs Road, Wolverhampton. Phosphorous was determined colorimetrically (Olsen’s method), magnesium and potassium were analysed using inductively coupled plasma optical emission spectrometry (ICP), and nitrogen was extracted with saturated calcium sulphate and analysed with an iron-selected electrode.

Plate 5.1 Combined provenance/progeny trial at Paradise Wood, Little Wittenham, Oxfordshire: a level site on former arable land at 50 m altitude. Walnuts planted in 75 cm treeshelters at 5 × 5 m.

Plate 5.2 Provenance trial at the Northwick Estate, Gloucestershire: an exposed hill top at 245 m altitude. Walnuts planted in 75 cm treeshelters at 4 × 4 m.

Plate 5.3 Provenance trial at the Maunsel Estate, Somerset: a sheltered small hill, sited above the low-lying Somerset levels at 15 m altitude. Walnuts planted in 75 cm treeshelters at 3.8 × 3.8 m.

sides, planted and protected in an identical manner to the trial trees to reduce the influence from edge effects. The guard row trees, supplied by a local tree nursery, were Hungarian in origin.

The trees on all three sites were planted during November and December 1998 and were protected with 0.75 m treeshelters, to prevent damage from grazing animals and herbicide spray drift. The decision to use these particular treeshelters was based on the conclusions of the walnut establishment trial described in Section 3.2. The systemic herbicide Glyphosate[3] was applied twice to ground vegetation surrounding the trees during the 1999 growing season.

5.2.2 Progeny trial

With increasing numbers of treatments (progenies) within an RCB design, block size must correspondingly increase in area and consequently the distance between plots will increase. Given the heterogeneity of most sites, however, the assumption that between-plot variance is the same for any two plots in a replicate becomes less reliable (Friedman and Namkoong 1986). The large number of progenies being tested in the field trials therefore required an appropriate means for accounting for field variation. Incomplete block designs are one such method where the objective is to construct a design in which any pair of treatments occurs equally often within a block (Cochran and Cox 1968). There are a number of recommended designs, all of which rely on some symmetry; *e.g.* lattice or cubic lattice designs (Cochran and Cox 1968). In forestry, however, balanced or partially balanced incomplete block designs have not been widely adopted because of the resulting restrictions on numbers of individuals, progenies and blocks that may be used (Friedman and Namkoong 1986). Unbalanced incomplete block designs, whose complicated analysis has been made possible with recent advances in computation (Friedman and Namkoong 1986), are well suited for progeny testing in forestry. For these reasons and after recommendation by Cox (pers. comm.) an unbalanced incomplete block design was therefore adopted for the progeny trial.

The progeny trial is located at the Oxfordshire site where it is combined with the provenance trial described above. The other provenance trial sites, having only four blocks each, would have yielded insufficient within-site replication and were therefore excluded from analyses. The progeny trial is an incomplete randomised block design of 14 blocks, each containing single tree plots for progenies. Any one progeny occurs only once in a block but because only

[3] 'Roundup Biactive Pro', Monsanto PLC. Applied at 4 l/ha in 1.2 m wide bands.

5-8 replicates were available for any progeny, none are present in all 14 blocks (Figure 5.2, p. 93).

The trial contains 199 half-sib progenies, each of which is replicated five to eight times, taken from 12 provenances (Table 5.4). The majority (95 %) of the progenies are from Kyrgyz provenances whilst the remaining progenies are from Spanish and Tajikistani provenances. The remaining 176 progenies of the 375 progenies included in the provenance trials are insufficiently replicated and are therefore excluded from the progeny trial. The identification of all progenies, even those excluded from statistical analyses has been maintained throughout the provenance/progeny trials. The location of each progeny within the trial and within blocks was randomised.

Table 5.4 Provenances and progenies represented in the progeny trial. The number of replications within progenies (*N*) are variable, as are the number of progenies nested within provenances (N_p).

																																SUMMARY
Provenance K2																																
Progeny	12	13	14	15	18	19	21	23	25	26	27																					N_p 11
N	6	7	6	5	6	5	5	6	7	6	6																					mean 5.91
Provenance K3																																
Progeny	31	32	33	34	35	36	37	38	39	40	41	42	46	47	49	50	51	52														N_p 18
N	6	6	6	6	5	5	5	5	5	7	5	6	7	6	6	6	5	6														mean 5.70
Provenance K4																																
Progeny	55	56	57	58	61	62	63	64	67	69	70	72	73	74	76	77	78	80														N_p 18
N	6	6	7	6	6	6	6	6	6	7	6	7	6	7	6	6	6	5														mean 6.17
Provenance K5																																
Progeny	81	82	84	85	86	88	90	91	92	93	94	96	97	98	99	100																N_p 16
N	6	6	6	6	7	6	6	7	7	5	6	6	5	6	6	6																mean 6.06
Provenance K6																																
Progeny	103	104	105	106	107	108	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126										N_p 22
N	7	5	7	5	6	5	6	6	6	6	6	6	6	6	6	6	6	5	6	6	6	7										mean 5.95
Provenance K7																																
Progeny	127	128	129	131	132	133	135	136	137	138	139	140	142	143																		N_p 14
N	7	6	6	6	6	6	7	7	8	6	7	7	7	7																		mean 6.64
Provenance K8																																
Progeny	145	148	149	150	151	152	154	155	157	158	160	161	162	163	164	165	166	167														N_p 18
N	6	6	6	6	6	6	6	6	6	7	6	6	6	6	7	6	7	6														mean 6.17
Provenance K9																																
Progeny	169	170	171	172	173	174	175	176	177	178	179	180	181	182	183	184	185	186	187	188	189	190	191	193								N_p 24
N	6	6	6	6	6	5	5	6	7	5	5	6	6	7	6	7	6	6	5	7	6	6	7	7								mean 6.04
Provenance K10																																
Progeny	194	195	196	197	198	199	200	201	202	203	204	205	207	208	210	211	212	213	214	215	216	217	218	219	220							N_p 25
N	6	7	5	6	6	6	6	6	6	7	5	7	7	6	6	6	6	7	6	6	6	6	6	6	6							mean 6.12
Provenance K11																																
Progeny	221	222	224	225	226	227	228	229	230	231	233	234	235	236	237	238	239	240	242	243	245	246	247									N_p 23
N	6	6	6	6	6	6	6	7	6	7	6	7	7	8	6	6	7	6	7	6	6	6	6									mean 6.35
Provenance E1																																
Progeny	248	249	250	251	252																											N_p 5
N	5	6	6	5	6																											mean 5.60
Provenance J1																																
Progeny	253	254	256	257	259																											N_p 5
N	6	5	6	5	5																											mean 5.40
																																TOTAL N_p 199
																																mean *N* 6.08
																																Total number of trees 1210

5.3 Assessments

The trees were measured for total height and stem diameter when they were planted in the winter of 1998. These measurements provide important information for later use, both as covariates in statistical analyses and in the assessment of growth, particularly in attempting to account for maternal effects and non-genetic variation (Burdon and Sweet 1976). Tree heights were measured to the nearest centimetre using a measuring rule whilst stem diameter was measured at the root collar using callipers, to the nearest millimetre. These measurements were repeated at the end of the first growing season, in October 1999, when tree survival was also recorded.

Flushing assessments were made on two days at the Oxfordshire site, Julian days 85 (March 26th 1999) and 97 (April 7th 1999), which were selected to represent the periods over which the greatest progression of flushing occurred. An additional assessment of flushing was made at the Gloucestershire and Somerset sites on day 120 (April 30th 1999). The scoring system adopted was similar to that used in the assessments of flushing in the establishment trial (Section 3.2) but simplified to cover the progression of bud burst only without recording shoot elongation (Figure 5.3).

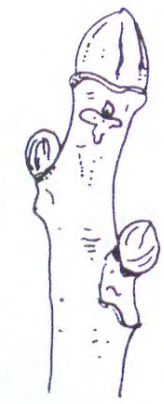
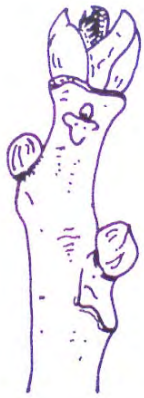
0	**1**	**2**
		
BUD CLOSED Scales closed	**BUD BREAKING** Scales separated and leaves visible	**BUD FLUSHED** Leaves extended beyond bud scales

Figure 5.3 Scoring system for assessing flushing in the walnut provenance/progeny trials.

5.4 Methods of analysis and results

5.4.1 Provenance trials

Plot summary files were computed using S-PLUS, with the assistance of Mario Cortina Borja[4], for heights and stem diameters for all provenances (Appendix V), although the greatest emphasis has been placed on the analysis of the 18 provenances (1-18) for which there was full replication across the three sites. The summary file includes plot means, variances and the number of trees-within-plots. This method, as recommended by Williams and Matheson (1994), had the advantage of reducing the very large raw data set into a manageable size. Increments of height (*IH*99) and stem diameter (*ID*99) were calculated from the main data set and then summarised by plots, as above.

SURVIVAL

Overall, survival across the three provenance trials was excellent with the loss of only 24 trees from the 2200 planted (98.9 % survival). Mortality was greatest at the Oxfordshire site with the loss of 21 trees although this figure only amounted to 1.50 % mortality within the site. On the two smaller trial sites, mortality rates were lower with the loss of only one tree at the Gloucestershire site (0.25 % mortality) and two trees at the Somerset site (0.50 % mortality).

As a result of the very high survival rates, differences in mortality between provenances, blocks or with sites were non-significant. However it was noted that the mean height (*H*98) and stem diameter (*D*98) of the trees that later died, was 7.3 cm and 6.1 mm respectively. These trees were well below the overall mean for these variables at planting time, *H*98 = 13.5 cm and *D*98 = 10.6 mm. Within the Oxfordshire site the mean *H*98 of all trees was 11.2 cm whilst those that died were on average only 7.3 cm. A *t* test for mean *H*98 and survival at the site confirmed a highly significant relationship between tree height at planting time and later mortality ($t = 2.9931$, $df = 1398$, $p=0.003$). Similarly, for mean *D*98 within the Oxfordshire site, the trees that died were significantly smaller (mean of 6.0 mm) than the total mean for *D*98 (9.9 mm) ($t = 3.9896$, $df = 1398$, $p<0.001$). There is no British Standard for minimum nursery seedling height and stem diameter for walnut as there is for other more commonly planted broadleaved trees (BS 3936: Part 4: 1984) (Morgan 1999). Minimum root collar diameters of 5 mm are recommended for 20 cm tall seedlings of oak, ash, cherry and lime (Morgan 1999). Aldhous (1972) recommended that walnuts should be planted as four-year-old (2+2) transplants, by which time they will be sturdy trees. It is well established that

[4] Dr. Mario Cortina Borja, Consulting and Teaching Officer, Department of Statistics, University of Oxford.

seedling size, especially root collar diameter, is an important criterion in nursery stock selection and subsequent seedling survival (Kerr and Evans 1993b).

The spatial distribution of mortality within the Oxfordshire site was examined in order to check for departures from randomness compared to clustering, using Ripley's *K* function (Ripley 1981). There was no evidence of significant deviations from a random pattern of mortality.

TREE HEIGHT

At planting time (December 1998), mean provenance heights (*H*98*)* were significantly (p=0.050) less at the Oxfordshire site than at the other two sites (Table 5.5a). This is explained by the absence of the minor provenances (19-25) in the Oxfordshire site, as confirmed by the analysis of provenances 1-18 only (Table 5.5b), where differences between provenance means for *H*98 are non-significant. At the end of the first growing season in autumn 1999, tree heights (*H*99) for the main provenances (1-18) were significantly smaller at the Gloucestershire compared to the Oxfordshire site. This is a result of the significantly (p=0.050) greater height increment (*IH*99) in the Oxfordshire site (Table 5.5b).

Table 5.5 Summary statistics for tree height based on plot means (*N*) for end of year tree heights in 1998 (*H*98) and 1999 (*H*99) and height increment 1998 to 1999 (*IH*99). Within each variable, figures followed by the same letter do not significantly differ from each other at p=0.050.

a. All provenances (1 to 25).

VARIABLE		SITE			ALL
		Oxfordshire	Gloucestershire	Somerset	SITES
		N = 254	*N* = 100	*N* = 100	*N* = 455
*H*98 (cm)	mean	12.11[a]	14.72[b]	15.61[b]	13.46
	s.e.	0.36	1.02	0.86	0.36
*H*99 (cm)	mean	47.91[a]	44.72[a]	53.68[b]	48.47
	s.e.	1.13	1.48	2.24	0.87
*IH*99 (cm)	mean	35.72[a]	29.99[b]	38.06[a]	34.96
	s.e.	0.97	1.93	1.93	0.74

b. 18 main provenances (1 to 18).

VARIABLE		SITE			ALL
		Oxfordshire	Gloucestershire	Somerset	SITES
		N = 252	*N* = 72	*N* = 72	*N* = 396
*H*98 (cm)	mean	12.13[a]	11.70[a]	13.22[a]	12.25
	s.e.	0.36	0.44	0.55	0.26
*H*99 (cm)	mean	47.95[a]	41.89[b]	47.19[ab]	46.71
	s.e.	1.13	1.43	2.32	0.88
*IH*99 (cm)	mean	35.76[a]	30.18[b]	33.96[ab]	34.41
	s.e.	0.97	1.18	2.03	0.76

An analysis of variance using the general linear model (GLM) for provenance height in 1999 (*H*99) was computed using plot means, with the statistical package S-PLUS. A Box-Cox transformation (Venables and Ripley 1997) of the height and increment of height data indicated that a square root transformation was appropriate. For stem diameters, the data were normally distributed but the square roots of values were again used, in this instance because it reduced variance. The basic model of analysis is shown in Table 5.6 (Model a) which includes provenance, site and block-within-site strata, and provenance × site interaction. The variation for *H*99 was highly significant ($p<0.001$) for all these strata except provenance × site interaction.

Table 5.6 Analysis of variance results for tree heights in 1999 (*H*99) for provenances 1-18 across three sites. Analyses were undertaken with plot means using the square root transformed values of tree heights.

Model a. Basic model of analysis.

	df	Sum of Sq	Mean Sq	*F* Value	*p* Value
Provenance	17	899.1817	52.89304	25.98188	0.00000000
Site	2	35.1356	17.5678	8.62957	0.00022350
Block-within- Site	19	226.1176	11.90093	5.84592	0.00000000
Provenance × Site	34	90.976	2.67576	1.31438	0.11934240
Residuals	322	655.5169	2.03577		

Model b. Model a with the addition of mean height in 1998 (*H*98) as a covariate.

	df	Sum of Sq	Mean Sq	*F* Value	*p* Value
Provenance	17	899.1817	52.893	33.3377	0.00000000
Site	2	35.1356	17.5678	11.0727	0.00002238
mean *H*98 (covariate 1)	1	218.6429	218.6429	137.8072	0.00000000
Block-within- Site	19	169.7294	8.9331	5.6304	0.00000000
Provenance × Site	34	74.9442	2.2042	1.3893	0.07882559
Residuals	321	509.294	1.5866		

Model c. Model b with addition of the quadratic term of mean *H*98 ($H98^2$) as a covariate.

	df	Sum of Sq	Mean Sq	*F* Value	*p* Value
Provenance	17	899.1817	52.893	35.2637	0.00000000
Site	2	35.1356	17.5678	11.7124	0.00001233
mean *H*98 (covariate 1)	1	218.6429	218.6429	145.769	0.00000000
mean $H98^2$ (covariate 2)	1	61.8045	61.8045	41.205	0.00000000
Block-within- Site	19	142.6633	7.5086	5.006	0.00000000
Provenance × Site	34	69.5231	2.0448	1.3633	0.09140106
Residuals	320	479.9767	1.4999		

The significance levels in the first model (a) were improved by including mean *H*98 as a covariate, as indicated in Table 5.6 (Model b). However, a plot of the square root of *IH*99

against the mean of *H*98 (Figure 5.4) reveals a quadratic structure where the larger trees in 1998 grew less in 1999 than trees with intermediate values for *H*98. Large seedlings are sometimes severely checked after planting although medium-sized trees usually retain an advantage over small seedlings (Burdon and Sweet 1976). Therefore, it was found that the model could be further improved, except for provenance × site interaction, by including the quadratic term of mean *H*98 ($H98^2$) as a covariate (Table 5.6 Model c). The effect of including these covariates is clear from the change in *p* values in the models presented in Table 5.6 but their values were quantified by computing an analysis of deviance (Table 5.7, p.106). There was a striking improvement by including *H*98 as a covariate (Model b) where, by loosing one residual degree of freedom, 146 residual sum of squares (*RSS*) were gained. Model b was further improved but by a lesser degree, in Model c by using the quadratic mean of *H*98 as a covariate with an overall gain of 176 *RSS*, but with the loss of only two residual degrees of freedom. Similar advantageous effects of including these covariates were found for diameter data, and increments of height and diameter data. Model c was therefore used in all analyses, unless otherwise specified.

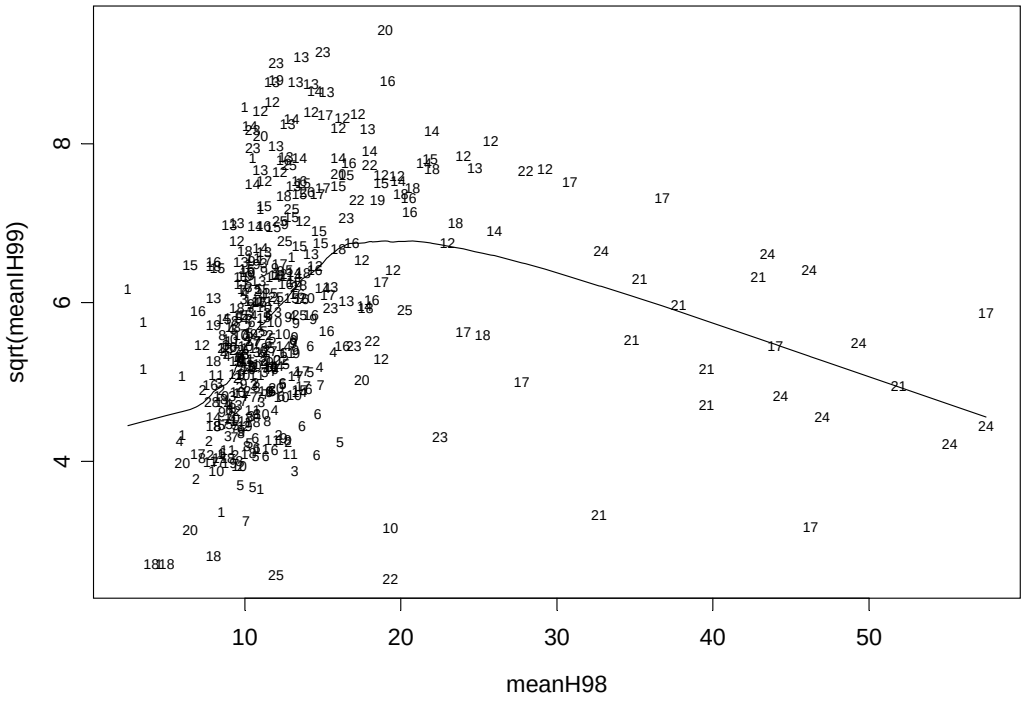


Figure 5.4 Plot of the square root of mean *IH*99 against mean *H*98 with a nonparametric smoother. Numbers (1-25) refer to provenances whose co-ordinates indicate plot means.

Table 5.7 Analysis of deviance for square root of *H*99 for models a-c in Table 5.6.

Model	Residual *df*	Residual Sum of Sq	Test covariate	*df*	Sum of Sq	*p* Value
a	322	655.5169				
b	321	509.2940	mean *H*98	1	146.2229	0.00000000
c	320	479.9767	mean *H*98 + mean $H98^2$	1	29.3173	0.00000006

Mean provenance heights in 1999 are listed in Table 5.8 although a simple interval plot is a valuable way of examining the results (Figure 5.5). Based on plot means across the three field trials (possible because a significant GEI was absent), tree heights for the main 18 provenances at the end of 1999 (*H*99), were more variable than at planting time (*H*98) to the extent that two groups were evident (Figure 5.5). One group, the Kyrgyz provenances (K1-11), are apparently shorter than the remaining seven provenances except T2.

Table 5.8 Overall and within-site mean provenance height (cm) in the autumn of 1999 (*H*99), based on plot means.

SITE	OXFORDSHIRE		GLOUCESTERSHIRE		SOMERSET		OVERALL	
PROVENANCE	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.
K1	44.2	5.5	32.8	7.1	30.1	2.8	39.4	3.8
K2	34.1	1.8	34.8	6.3	31.7	2.3	33.8	1.6
K3	41.6	1.8	34.7	2.9	34.9	2.2	39.1	1.5
K4	42.6	1.7	36.4	3.2	37.8	1.4	40.6	1.4
K5	39.2	2.2	33.2	3.1	34.5	5.1	37.3	1.8
K6	38.2	1.7	32.9	1.8	33.1	2.6	36.3	1.3
K7	37.1	2.0	42.0	2.3	34.9	2.5	37.6	1.5
K8	32.7	1.6	32.1	1.9	33.4	3.2	32.7	1.2
K9	44.9	2.2	40.8	2.9	43.7	6.5	44.0	1.8
K10	37.6	2.1	38.6	2.0	35.2	2.0	37.3	1.4
K11	39.1	2.2	34.0	3.0	37.4	4.9	37.8	1.7
E1	73.1	4.7	63.5	2.0	74.0	7.1	71.5	3.3
J1	71.3	4.6	62.4	5.6	70.1	12.5	69.4	3.7
P1	66.9	5.3	49.9	3.5	67.1	6.3	63.8	3.8
R1	60.5	2.8	51.1	3.5	58.4	10.6	58.4	2.6
S1	60.6	4.2	45.4	4.3	60.6	4.4	57.8	3.1
T1	52.1	5.0	43.3	7.3	83.4	4.8	56.2	4.5
T2	47.1	6.3	46.6	7.3	49.2	9.6	47.4	4.4
B1	NA	NA	37.1	5.5	61.2	12.9	48.0	7.1
B2	NA	NA	44.6	12.2	62.6	19.0	53.6	11.0
A1	NA	NA	67.7	3.1	69.0	8.6	68.4	4.3
F1	NA	NA	37.0	3.8	71.2	8.2	54.1	7.7
F2	NA	NA	51.5	5.5	86.2	6.1	68.8	7.6
F3	NA	NA	74.6	2.6	80.1	4.7	77.4	2.7
U1	NA	NA	45.3	9.6	62.3	3.8	53.8	5.8
OVERALL	47.9	1.1	44.7	1.5	53.7	2.2	48.5	0.9

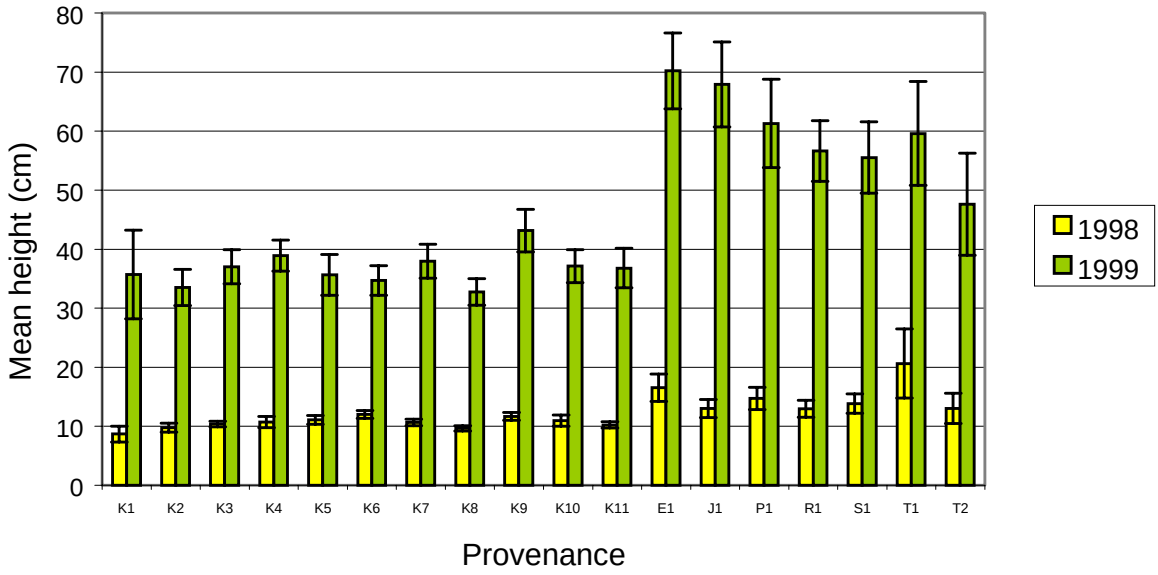


Figure 5.5 Mean tree heights for provenances in 1998 and 1999, based on plot means across the three field trial sites. Error bars show 95 % confidence limits.

Analysis of variance for *H*99 was calculated for some sub-groups of provenances to test whether any other underlying variation was present. For the Kyrgyz provenances (1-11) there was significant (p<0.001) variation between some of the provenances and for block-within-site (187 residual degrees of freedom). Variation for *H*99 was significant for sites (p=0.001) but non-significant for provenance × site interaction (p=0.794). Provenances 12 to 18, were also tested as a sub-group (112 residual degrees of freedom); there was significant (p<0.001) variation for provenance and site, and for block-within-site (p=0.011), although provenance × site interaction was non-significant (p=0.166). For the minor seed collections (19-25), only present on the Gloucestershire and Somerset sites (34 residual degrees of freedom), there was significant (p=0.006) variation for *H*99 between provenances and sites (p<0.001) but no significant variation either for block-within-site and no significant provenance × site interaction.

HEIGHT INCREMENT

Analysis of height increment from planting time in 1998 to the end of the first growing season in 1999 (*IH*99) is a more realistic reflection on tree performance within the field trials than is total height (*H*99). Figure 5.6 illustrates increments of height (*IH*99) for the main provenances within each site which indicates some notable variations both between provenances and for provenances-within-sites. The Kyrgyz provenances had similar vigour

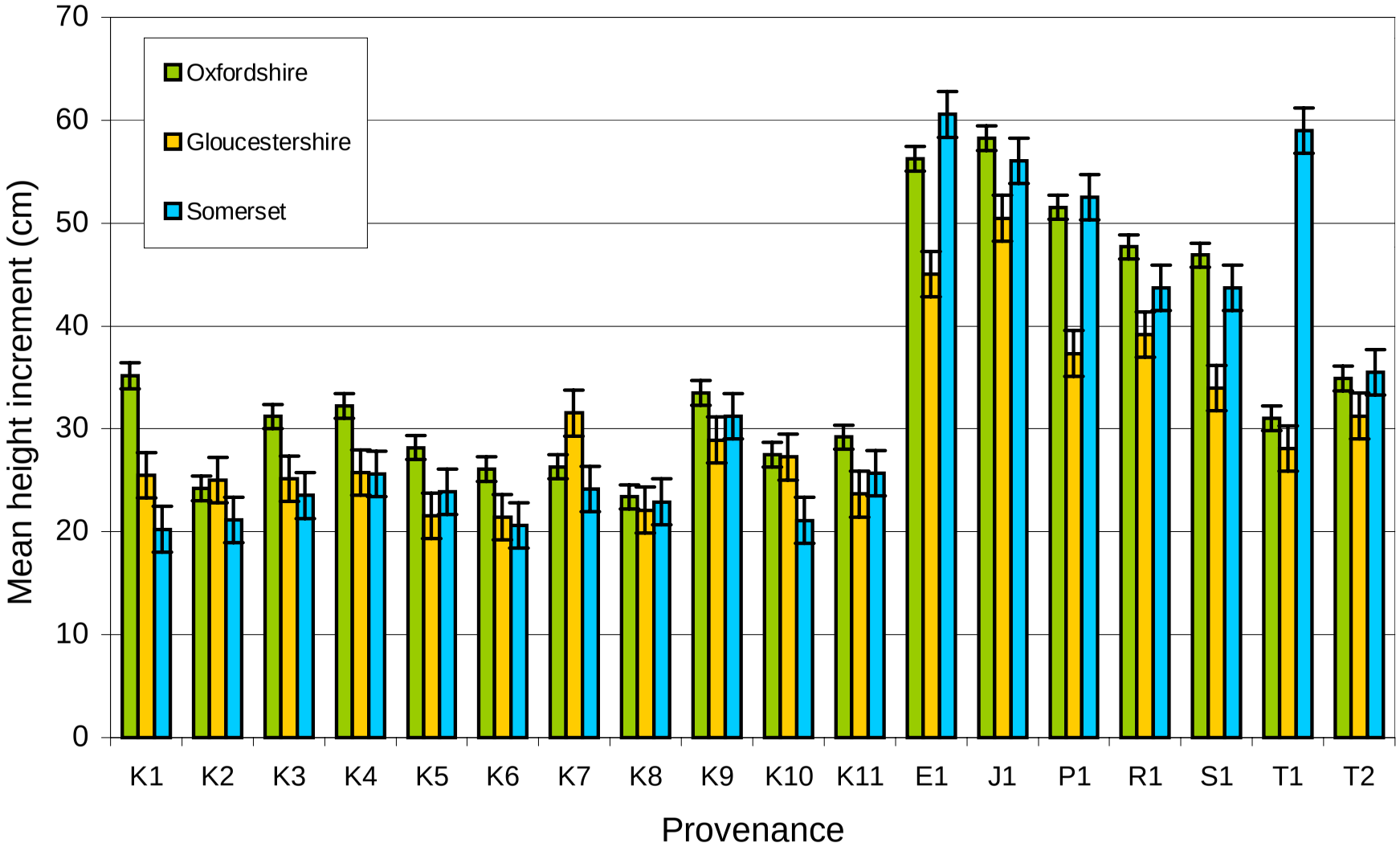


Figure 5.6 Mean height increment for provenances 1-18 in 1999, based on plot means within each of the field trial sites; Oxfordshire, Gloucestershire and Somerset. Error bars show 95 % confidence limits.

and performed best on the Oxfordshire site, with two exceptions (K2 and K7). In contrast, the remaining seven provenances appear to indicate more within-provenance × site interaction.

Mean height increment for the main provenances (1-18) was 34.4 cm (Table 5.5, p.103) which was encouraging given the small size and young age of the seedlings when planted. There were significant differences (p=0.050) between some sites for *IH*99 with most growth occurring on the Oxfordshire site (Table 5.5). A full model of analysis of variance was computed for *IH*99 and the main 18 provenances using the same method as for *H*99. The results indicate highly significant (p<0.001) differences for provenance, site and block-within-site but provenance × site interaction was marginally non-significant (p=0.052) (Table 5.9). Differences for provenance, site and block-within-site for the two sub-groups, provenances 1-11 and 12-18, were significantly different at p<0.001 but provenance × site interaction was non-significant for both sub-groups.

Table 5.9 Analysis of variance of height increment (*IH*99) for provenances 1-18 using the transformed (square root) values of *IH*99 and with plot means, and with two covariates: mean *H*98 and the quadratic term of mean *H*98 (*H*98^2).

	df	Sum of Sq	Mean Sq	*F* Value	*p* Value
Provenance	17	876.2078	51.54163	24.66642	0.00000000
Site	2	50.6082	25.30408	12.10984	0.00000852
mean *H*98	1	37.2273	37.22731	17.81598	0.00003174
mean *H*98^2	1	70.9733	70.97331	33.9659	0.00000001
Block-within- Site	19	196.1815	10.32534	4.94143	0.00000000
Provenance × Site	34	103.6241	3.04777	1.45858	0.05242418
Residuals	320	668.6548	2.08955		

Multiple comparison of means for the square root of *IH*99 were undertaken using Fisher's LSD procedure (Hoppe 1993) for the main provenances (1-18), with *N*99 as weighting and *H*98 as a covariate. The results of which statistically confirm the patterns visible in Figure 5.6 and support the use of the provenance sub-groups in the analysis of variance. All of the Kyrgyz (K1-11) and the two Turkish provenances (T1 – 2) grew significantly (p=0.050) less in height than provenances E1 and J1. Provenances P1, R1 and S1 had significantly (p=0.050) greater height increment than provenances K2-11. A multiple comparison of means (Fisher's LSD procedure) of the Kyrgyz provenances only, permitted a more precise assessment of variation within this sub-group. Provenance K9, which had the greatest height increment of the Kyrgyz provenances, grew significantly more (p=0.050) than K2, K5, K6, K8 and K10, whilst K4 grew significantly (p=0.050) more than K6 and K8.

Variation in height growth within each of the sites (Table 5.10) was explored using block means but there were no significant differences for *IH*99 within any of the sites. However, variation was present, although non-significant in a Tukey test, for *IH*99 between blocks at the Oxfordshire site. The two southern blocks 13 and 14 (Figure 5.2, p. 93) had mean *IH*99 values of 26.63 cm (s.e. 1.47) compared to a mean *IH*99 of 37.32 cm (s.e. 0.91) for the remaining 12 blocks. This phenomenon was further explored by conducting a spatial analysis of height increment within the Oxfordshire site.

Table 5.10 Overall and within-site mean provenance height increment (cm) 1998-1999 (*IH*99), based on plot means.

SITE	OXFORDSHIRE		GLOUCESTERSHIRE		SOMERSET		OVERALL	
PROVENANCE	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.
K1	35.2	5.2	25.5	6.1	20.2	2.8	30.5	3.6
K2	24.2	1.6	25.0	5.0	21.2	2.3	23.8	1.4
K3	31.2	1.7	25.1	2.7	23.5	2.8	28.7	1.4
K4	32.2	1.4	25.8	2.0	25.6	1.3	29.9	1.2
K5	28.2	1.9	21.6	2.8	23.9	5.1	26.2	1.6
K6	26.1	1.6	21.4	1.4	20.6	2.4	24.2	1.2
K7	26.3	1.9	31.5	2.6	24.2	1.9	26.9	1.4
K8	23.4	1.5	22.1	1.9	22.9	2.8	23.1	1.1
K9	33.5	2.1	28.9	2.3	31.2	6.4	32.2	1.8
K10	27.5	2.0	27.3	1.6	21.1	3.8	26.3	1.5
K11	29.2	2.1	23.7	2.5	25.7	5.3	27.6	1.7
E1	56.3	3.9	45.1	1.8	60.6	7.1	55.0	2.9
J1	58.3	4.2	50.5	5.1	56.1	11.6	56.4	3.4
P1	51.5	4.7	37.3	3.3	52.5	6.8	49.1	3.4
R1	47.7	2.1	39.2	2.4	43.7	8.6	45.4	2.1
S1	46.9	3.7	34.0	3.0	43.7	3.2	44.0	2.6
T1	31.0	3.3	28.1	4.6	59.0	3.8	35.6	3.3
T2	34.9	4.9	31.2	5.1	35.5	7.7	34.3	3.4
B1	NA	NA	28.2	5.4	48.5	12.1	37.2	6.5
B2	NA	NA	32.5	10.3	49.2	17.2	40.9	9.8
A1	NA	NA	26.2	1.8	31.9	7.0	29.0	3.5
F1	NA	NA	23.9	5.8	50.8	6.9	37.4	6.6
F2	NA	NA	33.6	6.6	74.2	5.2	53.9	8.6
F3	NA	NA	23.0	2.7	37.8	5.6	30.4	4.0
U1	NA	NA	32.6	9.4	47.8	5.2	40.2	5.7
OVERALL	35.7	34.9	30.0	29.7	38.1	36.9	35.0	0.7

A contour plot of *IH*99 for the Oxfordshire site was calculated, using S-PLUS, with the interpolation procedure known as kriging (Ripley 1981). Similar to conventional contouring algorithms, kriging is a linear interpolation method that allows the prediction of unknown values of a random function, from observations at known locations. A particular advantage of kriging is that it provides a measure of error associated with the contoured surface. The first

stage in creating the surface plot is to fit a theoretical semi-variogram that adjusted the model for the degree of spatial continuity. Using the spatial statistics module of S-PLUS (Kaluzny *et al.* 1998), a Gaussian semi-variogram was found to provide the best fit because the greatest variation in *IH*99 was in the local region. Isotropy, *i.e.* homogeneity of trends for any particular direction, was checked but no preferential directions were found, thereby allowing kriging to be used without any further adjustments to fit trend surfaces. Kriging uses the information from the semi-variogram to find an optimum set of weights which are then used to estimate the surface at unsampled points. Under the assumption of isotropy, the semi-variogram is a function of distance, therefore only the weighting changes according to the geographic arrangement of samples, which in this case was a precise grid of 5×5 m. The range of the kriging model was then specified for interpolated increments of height between 16 and 42 cm with contour intervals of 2 cm. The resulting surface contour plot is shown in Figure 5.7.

A declining trend in *IH*99 is clearly visible in Figure 5.7 in the region of the southern blocks 13 and 14. This correlates with the calculated block means for *IH*99 reported above. However, the standard error plot for this surface showed no significant trends although, given the non-significant differences between block means, this was expected. Nevertheless, the trends in *IH*99 indicated in Figure 5.7 were compared to detailed soil and subsoil maps of the site (Heming 1995), which indicated some interesting possible interactions. Blocks 13 and 14 lie on a subsoil boundary between well-drained river terrace gravel to the north and calcareous Gault clay to the south. The clay lies at approximately 120 cm depth and shows evidence of periodic anaerobic conditions indicating moderate to poor drainage. This can be supported by the author's personal experience of finding the soil to be more saturated in the two southern blocks, than the remainder of the trial, during tree planting in winter 1998. Good drainage is widely reported to be an important factor for successful walnut silviculture (Savill 1991). A further correlation between *IH*99 and pedology was hypothesised in comparing soil types at 50 and 80 cm depths. A slight 'ridge' in *IH*99 is evident mid-way across a north/south transect of the trial. This exactly coincides with a 'trench' of sandy clay that is found at around 80 cm depth running across the trial, which is deeper than the surrounding area where river terrace gravel is found at approximately 50 cm depth. Walnuts, grow best on deep soils, ideally in the range of 120 to 150 cm (Istvan and Tibor 1990).

The role of sub-blocks in the provenance trials, which were included as an insurance against significant within-block variation, was explored with an analysis of variance using within-

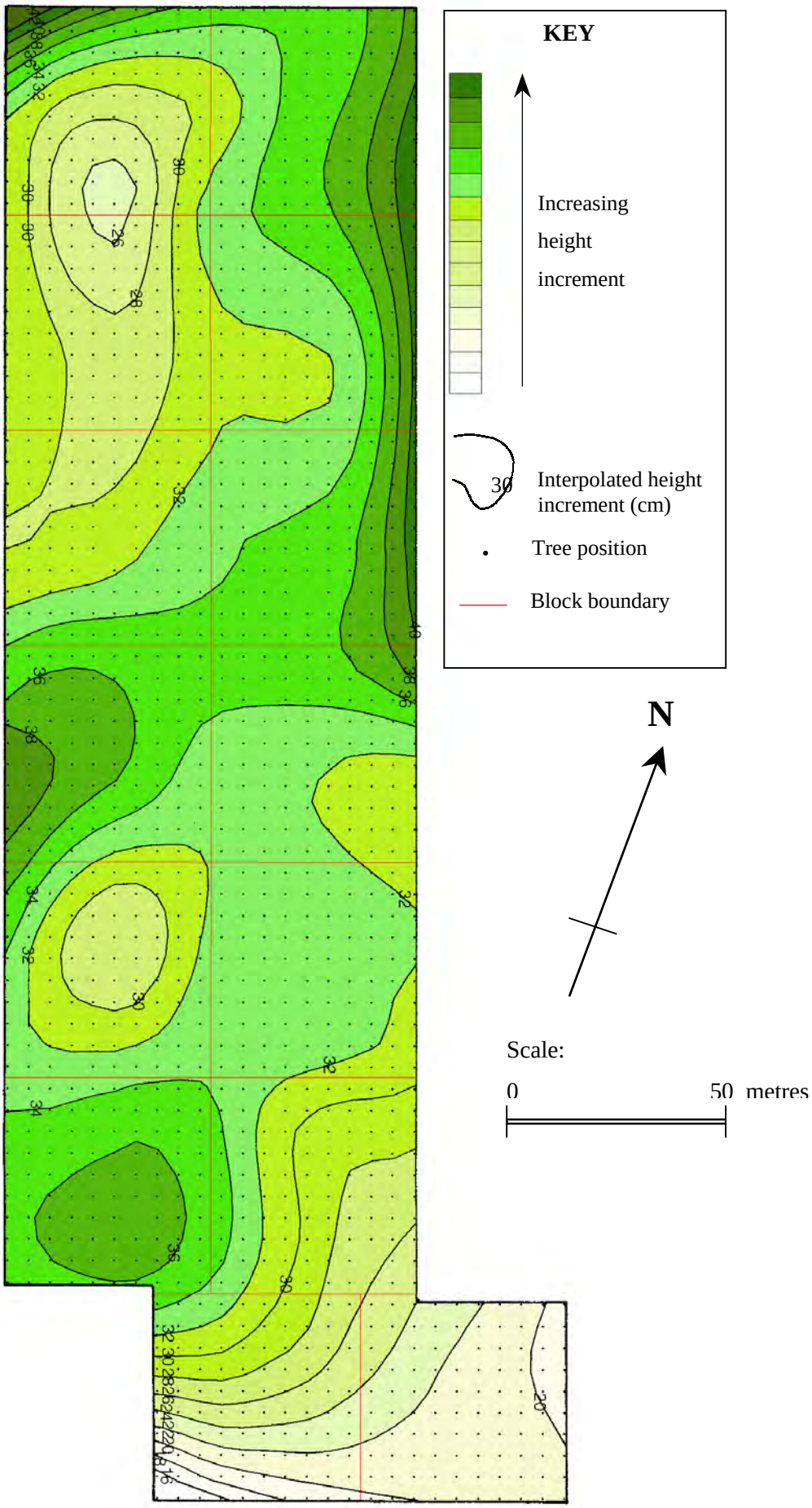


Figure 5.7 Kriged surface contour plot of tree height increment during 1999 in the walnut provenance/progeny trial, Little Wittenham, Oxfordshire.

block provenance means for increment (*IH*99 and *ID*99). This was only possible for the 10 provenances that were replicated four or more times within a block, hence having at least one replicate in each sub-block. Out of the 220 possible combinations (10 provenances by 22 blocks) there were few cases of significant differences at p=0.050 for a provenance within a block: for *IH*99 there were eight cases and for *ID*99, 16. Given the very small number of residual degrees of freedom in this analysis (maximum of seven) it can be concluded that there is no gain in incorporating the sub-blocks in the full model of analysis for provenance performance.

STEM DIAMETER

The summary statistics for stem diameters at the end of the growing season in 1999 (*D*99) (Table 5.11) indicate that trees in the Somerset site were significantly (p=0.050) greater than the other two sites, even though the significance values were calculated without the use of covariates. The full model of analysis for stem diameter (Table 5.12) indicates highly significant variation (p<0.001) for provenance, site and block-within-site but provenance ×site interaction is non-significant. There was significant (p<0.001) variation in *D*99 between provenances within the Kyrgyz sub-group and within the remaining provenances (12-18). As with the model for all 18 provenances (Table 5.12) there was no significant provenance × site interaction within either sub-group.

Table 5.11 Summary statistics for stem diameters based on plot means (*N*) for end of year diameters in 1998 (*D*98) and 1999 (*D*99) and diameter increment 1998 to 1999 (*ID*99). Within each variable, figures followed by the same letter do not significantly differ from each other at p=0.050.

a. All provenances (1 to 25).

VARIABLE		SITE			ALL
		Oxfordshire	Gloucestershire	Somerset	SITES
		N = 254	*N* = 100	*N* = 100	*N* = 455
*D*98 (mm)	mean	10.64[a]	9.90[a]	11.27[b]	10.61
	s.e.	0.22	0.36	0.32	0.16
*D*99 (mm)	mean	14.45[a]	14.98[a]	18.6[b]	15.48
	s.e.	0.22	0.35	0.45	0.19
*ID*99 (mm)	mean	3.72[a]	5.07[b]	7.31[c]	4.81
	s.e.	0.12	0.17	0.28	0.12

b. 18 main provenances (1 to 18).

VARIABLE		SITE			ALL
		Oxfordshire	Gloucestershire	Somerset	SITES
		N = 252	*N* = 72	*N* = 72	*N* = 396
*D*98 (mm)	mean	10.65[a]	9.34[b]	10.65[a]	10.41
	s.e.	0.22	0.36	0.32	0.16
*D*99 (mm)	mean	14.45[a]	14.44[a]	17.66[b]	15.03
	s.e.	0.22	0.33	0.46	0.18
*ID*99 (mm)	mean	3.72[a]	5.09[b]	7.00[c]	4.57
	s.e.	0.12	0.20	0.29	0.12

Table 5.12 Analysis of variance of stem diameter in 1999 (*D*99) for provenances 1-18. Analyses were undertaken using the square root transformed values of *D*99 using plot means, and with two covariates: mean *H*98 and the quadratic term of mean *H*98 ($H98^2$).

	df	Sum of Sq	Mean Sq	*F* Value	*p* Value
Provenance	17	75.94425	4.46731	31.8097	0.00000000
Site	2	40.19993	20.09996	143.1229	0.00000000
mean *H*98	1	85.91426	85.91426	611.7574	0.00000000
$H98 + H98^2$	1	0.72925	0.72925	5.1927	0.02334100
Block-within- Site	19	18.5308	0.97531	6.9447	0.00000000
Provenance × Site	34	5.18976	0.15264	1.0869	0.34522190
Residuals	320	44.9403	0.14044		

The largest mean stem diameters were attained by provenances A1 and F3 (Table 5.13), which was predictable as they were supplied as two-year old transplants (Table 5.1, p.92). A notable response to sites is found in the stem diameter (*D*99) for provenance T1, which at 24.4 mm in Somerset is almost twice the mean diameter of its trees in the Gloucestershire site (Table 5.13). A one-way analysis of variance for *D*99 of provenance T1 and site confirmed the significant variation for site at p=0.001 and in a Tukey test, trees at Somerset were shown to be significantly greater in stem diameter from those at the other two sites at p=0.010. Provenance T1 was also tallest (*H*99) at the Somerset site.

Variation in stem diameter increment was highly significant (p<0.001) within the 18 main provenances (Table 5.14). However, when the two sub-groups of provenances (1-11) and (12-18) were analysed separately, there were no significant differences within each sub-group thereby indicating that these two groupings account for this variation.

Multiple comparisons of *ID*99 means for provenances 1-18 were undertaken using Fisher's LSD procedure (Hoppe 1993), using the square root of *ID*99, with the plot count (*N*99) as weighting and *H*98 as a covariate. There were few significant differences at p<0.050 between provenances; and these were for the provenances with the least stem diameter increment, Kyrgyz provenances 2, 5, 6, and 8, in comparison to provenance R1 which had the largest mean of *ID*99. Within the Kyrgyz provenances (1-11), which were separately analysed to allow a more precise comparison within the sub-group, there were no significant differences for *ID*99.

Table 5.13 Overall and within-site mean provenance stem diameter (mm) in 1999 (*D*99), based on plot means.

SITE	OXFORDSHIRE		GLOUCESTERSHIRE		SOMERSET		OVERALL	
PROVENANCE	mean	s.e.	mean	s.e.	mean	s.e.	mean	s.e.
K1	13.2	1.2	13.0	2.4	16.3	0.9	13.7	0.9
K2	12.6	0.4	13.0	1.3	16.0	0.6	13.3	0.4
K3	13.5	0.3	14.1	1.1	15.6	0.4	14.0	0.3
K4	13.8	0.5	12.6	0.6	17.7	0.6	14.3	0.5
K5	13.1	0.4	13.6	0.4	14.5	1.1	13.4	0.3
K6	13.5	0.5	13.0	0.5	15.1	1.0	13.7	0.4
K7	12.8	0.4	13.9	0.1	15.4	0.6	13.5	0.4
K8	11.7	0.3	12.6	0.3	15.8	1.1	12.6	0.4
K9	13.8	0.4	14.5	0.6	16.8	1.8	14.5	0.5
K10	12.6	0.4	15.0	0.5	15.8	0.7	13.6	0.4
K11	12.7	0.4	13.2	0.8	15.9	1.4	13.4	0.4
E1	18.5	1.3	17.5	1.0	19.9	1.9	18.6	0.9
J1	16.9	1.0	16.6	0.7	20.9	3.0	17.6	0.8
P1	17.1	1.1	16.5	0.8	20.6	1.3	17.7	0.8
R1	16.5	1.2	16.3	1.1	19.1	2.9	16.9	0.9
S1	15.9	0.6	14.4	0.8	18.4	1.5	16.1	0.5
T1	16.2	0.8	13.8	2.4	24.4	2.3	17.3	1.0
T2	15.6	1.8	16.5	3.3	20.0	2.8	16.6	1.4
B1	NA	NA	13.5	1.3	16.5	1.0	15.5	0.8
B2	NA	NA	12.0	1.9	22.3	5.1	17.1	3.2
A1	NA	NA	20.3	0.4	23.9	2.1	22.1	1.2
F1	NA	NA	12.3	0.9	19.2	0.6	15.7	1.4
F2	NA	NA	15.5	0.9	20.6	2.4	18.1	1.5
F3	NA	NA	25.1	1.2	25.3	1.2	25.2	0.8
U1	NA	NA	15.6	0.6	19.2	0.7	17.4	0.8
OVERALL	14.5	0.2	15.0	0.4	18.6	0.4	15.5	0.2

Table 5.14 Analysis of variance of stem diameter increment 1998-1999 (*ID*99) for provenances 1-18. Analyses were undertaken using the square root transformed values of *ID*99 using plot means, and with two covariates: mean *H*98 and the quadratic term of mean *H*98 ($H98^2$).

	df	Sum of Sq	Mean Sq	*F* Value	*p* Value
Provenance	17	28.4719	1.67482	3.4333	0.00000740
Site	2	129.5574	64.77871	132.7935	0.00000000
mean *H*98	1	33.1519	33.15192	67.96	0.00000000
$H98 + H98^2$	1	0.1241	0.12405	0.2543	0.61441480
Block-within- Site	19	64.427	3.39089	6.9512	0.00000000
Provenance × Site	34	15.8826	0.46714	0.9576	0.53967400
Residuals	316	154.1497	0.48782		

PROVENANCE FLUSHING

Analysis of the flushing data for provenances was computed on plot means by S-PLUS using the Kruskal-Wallis non-parametric test (Venables and Ripley 1997). Provenance assessments for flushing at the Oxfordshire site on days 85 (March 26th 1999) and 97 (April 7th 1999) captured the main progression of bud burst for most provenances (Figure 5.8). On day 85 only three of the provenances (E1, T1 and T2) had progressed beyond a median score of 0.5, and T2 had a median score of 1.0 (K = 74.5314, df = 17, p<0.001).

Differences between provenances remained significant (p=0.001) when provenance T2 was removed from the analysis but when all three outlying provenances (E1, T1,and T2) were removed, there were no significant differences between provenances (K = 21.9413, df = 14, p=0.079).

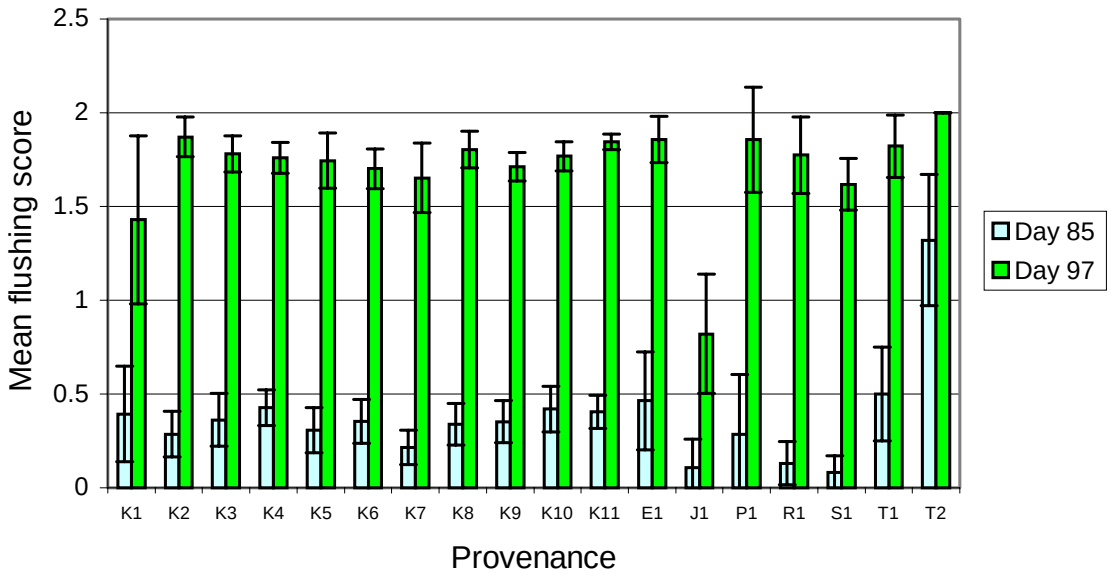


Figure 5.8 Provenance flushing at the Oxfordshire site on Julian days 85 (March 26th) and 97 (April 7th), 1999 (based on plot means). Error bars show 95 % confidence limits. (Details of what the scores indicate are shown in Figure 5.3: 0=bud closed, 1=bud breaking, 2=bud flushed.)

This result indicates that on day 85, these three provenances were accounting for all the variation in flushing between provenances. Within the Kyrgyz provenances, there was therefore no significant variation, although provenance K7 had the lowest mean flushing score at 0.21 (Figure 5.8).

On day 97, most provenances had almost completed flushing (Figure 5.8), having median scores exceeding 1.5, except provenances K1 and J1 (K = 92.976, df = 17, p<0.001). A rerun of the analysis excluding provenance J1 still indicated significant differences between provenances (K = 29.4594, df = 16, p=0.021). With provenances K1 and J1 removed, there was no significant (p=0.094) variation in flushing between the remaining provenances.

Analysis of flushing assessments for trees at the Gloucestershire and Somerset sites on day 120, using the Kruskal-Wallis test, indicated significant (p<0.001) variation between provenances (based on plot means). However, all of the significant variation was accounted for by provenances F1, F2 and F3 which all had medians of 0.0. With these three provenances excluded from the analysis, there was no significant variation in flushing between the remaining provenances (p=0.447). There was no significant variation in flushing between the two sites.

5.4.2 Progeny trial

The 1210 trees in the progeny trial represent approximately half the number of trees that would be present were a fully balanced progeny trial available (Table 5.15). Statistical analyses could be undertaken using the unbalanced data without difficulty, which is a tribute to the power of modern computers and sophisticated statistical packages.

Table 5.15 The ideal model of progeny trial design versus the actual model which has unbalanced replication between and within progenies.

	Provenances		Progenies (within provenances)		Replicates	TOTAL *N* trees
IDEAL	12	×	25	×	8	= 2400
ACTUAL	12	×	mean 16.58 range 5 - 25	×	mean 6.08 range 5 - 8	≅ 1210

There would almost inevitably be significant variation between progenies for the measured growth variables because of the large number of them, and indeed this is reflected in the provenance results presented in the previous section. Analyses of variance for stem heights and diameters were therefore not computed. The major application of data from the progeny trial was in assessing flushing variation, as described in Section 5.4.1, between the progenies and in developing genetic concepts and heritability estimates.

PROGENY FLUSHING

Flushing assessments were undertaken using the method described in the previous section for provenances, although in this case separate plot means based on amalgamated single-tree plots were computed for progeny assessments.

Progeny assessments (for the 199 progenies with five or more replicates) for flushing revealed that on day 85 (March 26th 1999), only 15 % of the progenies (31) had reached or exceeded a mean score of 1.0 (Table 5.16). The maximum mean score for flushing was 1.3. One (progeny 251, provenance J1) had attained a median score of 2.0 whilst three progenies exceeded a score of 1.0 with median scores of 1.5; progenies 77 (K4), 114 (K6) and 246 (K11). The Kruskal-Wallis test results on progeny flushing, using progeny medians, indicated significant differences between progenies (K = 301.6305, df = 198, p<0.001), and after excluding the four progenies whose median scores exceeded 1.0, the analysis remained significant (K = 280.8811, df = 194, p<0.001).

Table 5.16 Flushing scores for earlier flushing progenies on day 85 (March 26th 1999).

Progeny	Provenance	frequency	median	mean	range	std. dev.
251	J1	5	2	1.20	2	1.10
77	K4	6	1.5	1.33	2	0.82
114	K6	6	1.5	1.17	2	0.98
246	K11	6	1.5	1.33	2	0.82
19	K2	5	1	0.80	2	0.84
35	K3	5	1	0.60	1	0.55
36	K3	5	1	0.80	2	0.84
46	K3	7	1	0.86	2	0.69
51	K3	5	1	0.60	1	0.55
58	K4	6	1	0.83	1	0.41
72	K4	7	1	0.57	1	0.53
80	K4	5	1	0.60	1	0.55
96	K5	6	1	1.00	2	0.63
99	K5	6	1	0.83	1	0.41
116	K6	6	1	1.00	2	0.63
126	K6	7	1	0.86	2	0.90
167	K8	6	1	1.00	2	0.63
169	K9	6	1	1.17	2	0.75
170	K9	6	1	0.67	1	0.52
171	K9	6	1	0.83	2	0.75
178	K9	5	1	0.60	1	0.55
184	K9	7	1	0.86	1	0.38
194	K10	6	1	0.67	1	0.52
212	K10	6	1	0.67	1	0.52
214	K10	6	1	0.83	1	0.41
216	K10	6	1	0.67	1	0.52
218	K10	6	1	0.67	1	0.52
220	K10	6	1	0.67	1	0.52
228	K11	6	1	0.83	2	0.75
236	K11	8	1	0.63	1	0.52
248	E1	5	1	0.80	1	0.45

The remaining 168 progenies, with scores less than 1.0, showed no significant (p=0.810) differences for flushing. Consistent with the provenance results reported above, no progenies

within provenance K7 had attained a score of one on day 85. The provenances with the highest number of progenies that had attained scores ≥ 1.0 were from provenances K9 and K10, which had five and six progenies respectively.

On day 97 (April 7th 1999), only 12 progenies remained with median flushing scores ≤ 1.0 (Table 5.17) (K = 298.3968, df = 198, p<0.001) and with the four progenies with median scores < 1.0 excluded, differences remained significant (K = 228.6123, df = 194, p=0.045).

Table 5.17 Flushing scores for later flushing progenies on day 97 (April 7th 1999).

Progeny	Provenance	frequency	median	mean	range	std. dev.
104	K6	5	1	1.20	2	0.84
105	K6	7	1	1.29	2	0.76
129	K7	6	1	1.33	1	0.52
161	K8	6	1	1.17	2	0.75
173	K9	6	1	1.00	2	1.10
182	K9	7	1	1.14	2	0.90
207	K10	7	1	1.29	2	0.76
254	J1	5	1	1.00	2	1.00
190	K9	6	0.5	0.67	2	0.82
256	J1	6	0.5	0.50	1	0.55
253	J1	6	0	0.67	2	1.03
259	J1	5	0	0.00	0	0.00

Inevitably, there was a wider spread of scores for progenies on the later day. As the above results indicate, the four progenies with low scores account for most of this variation and once the progenies with median scores ≤ 1.0 were removed, there were no significant differences between progenies.

5.5 Genetic concepts and heritability estimates

Sources of variation in provenance/progeny trials may be explained by a linear model in terms of variance components, where these can be used to estimate genetic variation (Falconer and Mackay 1996). In the linear model of analysis for the walnut progeny trial, the only stratum variance component is at the plot level (the variance between two trees in different plots), as with single-tree plots there is no independent estimate of the within-plot variance component.

In multiple-tree plot experiments (with unbalanced within-plot replication), the residual mean square (s^2) from the analysis of plot means and the within-plots level variance component ($\hat{\sigma}_t^2$) is used to estimate the plots level variance component ($\hat{\sigma}_m^2$):

$$\hat{\sigma}_m^2 = s^2 - \frac{1}{\overline{w}}\hat{\sigma}_t^2$$

where $\overline{w}$ is the harmonic mean of the tree counts (Cochran and Cox 1968). In this case however, as $\hat{\sigma}_t^2$ is zero:

$$\hat{\sigma}_m^2 = s^2$$

The treatment variance component for the walnut progeny trial is for progeny-within-provenance (family) (σ_f^2) variance. This was calculated from a mixed-effects model (Vonesh and Chinchilli 1997) where provenances were fixed, and families were specified as random effects. Parameter estimation was undertaken using the residual maximum likelihood (REML) method in the statistical package S-PLUS (Venables and Ripley 1997). The model is written as:

$$Y_{ij} = X\tau + Z\xi_i + \eta_{ij}$$

where $i = 1_1\ldots, n_f$ families, $j = 1_1\ldots, n_p$ provenances, τ = vectors of fixed effects for provenances, X and Z = design matrices and Y_{ij} = height for family i in provenance j. η_{ij} = the error term for family i in provenance j, and var $(\eta) = \sigma^2 I + \sigma_b^2 ZZ'$ where σ^2 = fixed effects and σ_b^2 = random effects. In REML, the variance components σ^2 and σ_b^2 are estimated by iteration and then substituted in the expression for var (η). The parameters τ and random effects term ξ in the above model are obtained using generalised least squares (Patterson and Thompson 1971). The output also includes plot means for families where these values are known as 'best linear unbiased predictors' (BLUPs) (Robinson 1991), and these can be used for selection in breeding programmes.

The next stage in estimating heritability is to quantify the variation of the experimental trees from which parents could be selected for a future breeding programme (Williams and Matheson 1994). This is the phenotypic standard deviation (σ_p) which is the square root of the phenotypic variance (σ_p^2):

$$\sigma_p^2 = \sigma_f^2 + \sigma_m^2$$

where σ_f^2 = family variance component and σ_m^2 = plots level variance component.

HERITABILITY

Heritability (h^2) is the fraction of the variance in a given trait that is due to the additive effects of genes, the remainder being due to environment (Hazel and Lush 1942). Heritability for half-sib families (family heritability; h^2) is expressed:

$$h^2 = \frac{\sigma_f^2 / r}{\sigma_p^2}$$

where r = coefficient of relationship of each seedling with each other seedling in the same family (Falconer and Mackay 1996). For open-pollinated families, r is $^1/_2$ for full-sibs but equal to $^1/_4$ if they are strictly half-sib, *i.e.* when all of them were pollinated by unrelated males and none resulted from self-pollination (Squillace 1974). However, in natural populations these conditions are rarely fulfilled as there is likely to be some family structure and the actual value will lie between these two proportions; for example, there may be some shared or related paternal genes or some self-pollination. Heritability estimates based on the assumption that r is $^1/_4$ usually results in an overestimate of additive genetic variance (Namkoong 1966). Squillace (1974) estimated an average coefficient for open-pollinated seed from unrelated pines to be $^1/_3$, whilst Williams and Matheson (1994) used $^1/_{2.5}$ for natural eucalypt populations. The mating system of the genus *Juglans* is heterodichogamous, in that its mating type can be either protandrous (male gametes shed before female gametes mature) or protogynous (female gametes mature before male gametes) (Gleeson 1982). Heterodichogamy reduces the selective pressure against within-type separation, thereby allowing individuals to completely separate the timing of male and female flowering (Gleeson 1982). Available data for *J. hindsii* L. suggests that there may be very little selfing in this species, with only 4.6 % of male flowers overlapping flowers on protogynous individuals (Gleeson 1982). Wood (1934) reported considerable more overlap in flowering amongst domestic varieties of *J. regia*, although this would be the expected result of artificial selection for increased nut production (Gleeson 1982). Malvolti *et al.* (1995) estimated an outcrossing rate (t) of 0.977 which was not significantly (p<0.050) different from complete outcrossing (t=1). Estimations of genetic correlation in walnut are complicated by the fact that some individuals or varieties can reproduce asexually by apomixis (Loiko 1990). A correlation coefficient of $^1/_3$ is used in all calculations hereafter.

Table 5.18 summarises estimated variance components and family heritability estimates for height (*H*99) of families in the progeny trial. The estimated family variance component (σ_f^2) is effectively zero for two provenances, K8 and J1, at 7.02×10^{-21} and 1.62×10^{-28} respectively. It is possible to test the hypothesis of homogeneity using the variance components when the effects are random, as would be the case of fixed effects using the homogeneity of means (Snedecor and Cochran 1989). It is therefore possible to have infinitesimally small variances, due to sampling error consistent with the null hypothesis $\sigma_f^2 = 0$.

Table 5.18 Variance components and heritability estimates for height (*H*99): σ_f^2 = family variance component, σ_m^2 = plots level variance component, σ_p^2 = phenotypic variance component, σ_p = phenotypic standard deviation, h^2 = family heritability, h_w^2 = within-family heritability, σ_h^2 = standard of error of family heritability, and n = mean number of trees per family. See text for methods of computation.

Provenance	σ_f^2	σ_m^2	σ_p^2	σ_p	h^2	σ_h^2	h_w^2	n
K2	35.43	222.60	258.03	16.06	0.41	0.10	0.32	5.91
K3	50.05	262.23	312.28	17.67	0.48	0.09	0.38	5.70
K4	16.26	353.76	370.01	19.24	0.13	0.06	0.09	6.17
K5	15.15	290.14	305.29	17.47	0.15	0.07	0.10	6.06
K6	49.93	261.24	311.17	17.64	0.48	0.08	0.38	5.95
K7	25.35	245.62	270.97	16.46	0.28	0.08	0.21	6.64
K8	0.00	237.41	237.41	15.41	0.00	0.05	0.00	6.17
K9	5.72	391.38	397.10	19.93	0.04	0.05	0.03	6.04
K10	15.99	285.57	301.56	17.37	0.16	0.06	0.11	6.12
K11	13.09	280.84	293.93	17.14	0.13	0.05	0.09	6.35
E1	67.92	450.15	518.06	22.76	0.39	0.16	0.30	5.60
J1	0.00	470.89	470.89	21.70	0.00	0.12	0.00	5.40
Total	19.97	298.94	318.91	17.86	0.19	0.02	0.13	6.08

Within-family heritability (h_w^2) is calculated:

$$h_w^2 = h^2 \frac{(1-r)}{(1-t)}$$

where r = $^1/_3$, and *t* is the correlation of phenotypic values of members of the families $(\sigma_f^2 / \sigma_p^2)$ (Falconer and Mackay 1996).

A measure of the reliability of h^2 estimates is important although the calculation of the standard error of heritability is complex (Falconer and Mackay 1996). Wright (1976) provided an approximate method, which is only strictly true in simple cases where parents are selected randomly and the offspring planted in single-tree plots. The standard error of family heritability (σ_h^2) from Wright (1976) was calculated:

$$\sigma_h^2 \cong \frac{(1-t)(1+NBSt)}{\sqrt{[(NBS)(F-1)/2]}}$$

where *t* is the intraclass correlation ($^1/_4$ of single tree heritability), *NBS* is the number of trees per family and *F* is the number of trees. Standard error estimates for family heritabilities were calculated with the above formula and are presented in Table 5.18.

Calculation of *t*-ratios permitted a simple assessment of the significance level of variation for heritability estimates of families within provenances, and where $t \geq 1.96$ the heritability estimate is significant at $p=0.050$:

$$t = \frac{h^2}{s.e.(h^2)}$$

where h^2 = heritability estimate, and s.e. = standard error of family heritability. Other than for families within provenances K8 and J1, whose family variance component equalled zero, and families within provenance K9 ($t=0.86$), heritability estimates for families within the remaining provenances were significant at $p=0.050$ (Table 5.18). Heritability estimates vary considerably between families although, as Zobel and Talbert (1984) pointed out, such estimates should be viewed as figures that give a general indication of heritability and not as absolute values.

The family heritability estimate for walnut tree height at the Oxfordshire site was 0.19 (s.e. 0.02) (Table 5.18). In a summary of several studies, Zobel and Talbert (1984) concluded that heritability estimates in forest trees vary with species, populations within species, age, and characteristics assessed. The heritability estimate calculated above cannot therefore be compared, in an empirical sense, with other studies. Some characteristics, such as specific wood gravity, appear to be strongly controlled genetically whilst other traits, like height growth, are under a lesser control (Zobel and Talbert 1984). For example, heritability estimates for vigour in oak are 'probably low' but are high for several wood characteristics, such as density (0.65) and width of early wood ('high') (Savill and Kanowski 1993). Rink (1987) reported narrow-sense heritability estimates for timber characteristics of *Juglans nigra*: heritability of heartwood area was 0.56 and sapwood area was 0.40. Heritability estimates of height of broadleaved species vary considerably: for *Fraxinus excelsior* on four different sites they ranged from 0.16 to 0.52 at age 5 (Savill *et al.* 1999) whilst for *Platanus occidentalis* L. at age 5, the estimate of height heritability was 0.26 (Ferguson *et al.* 1977). Heritability estimates may change over time, both as the trees mature and as the environment changes, *e.g.* when competition between trees begins (Zobel and Talbert 1984), so caution should therefore be exercised in assessing young seedlings as they are likely to be greatly influenced by environmental factors (Stonecypher 1966). For example, McKeand (1978) reported a two-fold increase in heritability estimates in height growth of black walnut progenies from one year-old seedlings (0.55) to eight year-old trees (1.25). Rink (1984) estimated heritability estimates for black walnut height growth over 10 years and demonstrated that for the first four years after planting, heritability decreased rapidly but thereafter gradually increased and was expected to continue to do so until the next stage of

plantation development. Bresnan *et al.* (1994) reported provenance heritability estimates for height of 22-year-old black walnut trees, which ranged from 0.55 to 0.79 between different sites.

SELECTION AND GAIN

Estimates of family heritability facilitate the prediction of a response to selection among the progeny. The most common form of selection in advanced tree improvement programmes is family plus within-family selection, which is effective for low heritabilities (Zobel and Talbert 1984). This is a two-stage method where, firstly selections are made on families for a given parameter followed by a further selection of the best individual trees within these families. This relatively simple method can be improved by using combined selection by calculating an index (Falconer and Mackay 1996).

To illustrate a predicted response in tree height to a future thinning of the progeny trial, using the family plus within-family selection method, the following scenario was formulated. The decision to convert the progeny trial into a breeding seedling orchard is taken at year 20 with the first selections made on family performance, with the resulting culling of 91 families (Table 5.19). Twenty years later when the trees are 40 years old, the trees are thinned again to achieve a satisfactory stocking rate of 100 trees per hectare by keeping 4 individuals per family, leaving approximately 430 trees in 4.3 ha.

Table 5.19 Selection method for tree height in 1999 (*H*99) used in the calculation of genetic gain (see text for details).

Year		Number of families	Number of individual trees	Mean *H*99 (cm)
0	Trial prior to thinning	199	1210	40.76
20	Family selection (46 % culled)	108	657	48.24
40	Within-family selection (best 4 trees remaining per family)	108	432	56.90

The proportion selected is therefore a reduction of 199 families to 108, followed by six trees per progeny to four, which equates to proportions of 0.54 and 0.66 respectively. These values are used to estimate the selection intensity (*i*) with reference to the table of selection intensities in Becker (1984) for an infinite population size (Table 5.20).

Under family selection, the criterion for selection is the mean phenotypic value of the members of a family, so the expected response to family selection is:

$$R_f = i\sigma_p h^2 \frac{1+(n-1)r}{\sqrt{n\{1+(n-1)t\}}}$$

where i = the intensity of selection, σ_p = the standard deviation of phenotypic values of individuals, and h^2 = the heritability of family means (Falconer and Mackay 1996). Similarly, the expected response to within-family selection is:

$$R_w = i\sigma_p h^2 (1-r)\sqrt{\left[\frac{n-1}{n(1-t)}\right]}$$

where i = selection intensity, σ_p = standard deviation of phenotypic values of individuals, h^2 = heritability of individual values, r = coefficient of relationship ($^1/_3$), n = number of individuals in the families and t = correlation of phenotypic values of members of the families $(\sigma_f^2 / \sigma_p^2)$ (Falconer and Mackay 1996).

Table 5.20 Values used in the formulae to estimate response to selection (*R*) for height (*H*99) (after Falconer and Mackay 1996). See text for explanation of symbols.

Selection method	σ_p	h^2	t	n	i	r	Response to selection (*R)* (cm)
Family selection	17.858	0.188	0.063	6.08	0.732	0.333	R_f = 2.34
Within-family selection	17.858	0.188	0.063	4	0.558	0.333	R_w = 1.05

The estimated response to family selection (R_f), using the parameters presented in Table 5.20, is a gain of 2.34 cm (5.73 %) in tree height above the experimental mean (40.76 cm) (Table 5.19). The estimated response to within-family selection (R_w), in a further stage, predicts a gain of 1.05 cm in height (2.57 %). A gain projection based upon selecting the trees as detailed above therefore indicates a possible combined improvement of 3.39 cm (8.32 %) over mean unselected tree height.

These estimates should be viewed with caution as collections made from wild populations are likely to contain some structure, including a degree of inbreeding, which can inflate heritability estimates (Williams and Matheson 1994). In addition, there is no estimate of the increased gain that might be achieved from outcrossing, both between and within provenances and also, estimates of heritability will be more useful after one generation of outcrossing in the trial because they will more accurately reflect genetic differences (Williams and Matheson 1994). Savill *et al.* (1997) advocated that 30 - 50 % gains in the amount of recoverable wood may be possible from the first generation of breeding programmes, but perhaps half of this

gain would be in terms of improved height/diameter growth and half in terms of improved form (Savill, pers. comm.). Heritability, hence genetic gain, may change with age in the field trials, as demonstrated by the two-fold increase in heritability between one-year and eight-year-old trees reported by McKeand (1978) for black walnut.

5.6 Discussion and conclusions

The identification of superior genotypes is possible at an early stage in a field trial but only if differences in juvenile growth are strongly associated with eventual desired growth traits (Burdon and Sweet 1976). Additionally, genetic factors must be separated from non-genetic factors by decreasing the latter through adopting good experimental design and using covariates in the analyses (Burdon and Sweet 1976). It has been repeatedly stated above that caution should be exercised in assessing any of the measured traits or estimated parameters at this time because the material is so young. However, the results presented above indicate that some considerable and indeed significant variation exists in the collected genotypes for most of the traits assessed.

With regard to the main objectives of the field trials (Figure 5.1, p. 89), the early results presented here have provided limited scope for assessments based on correlating growth traits with either provenance origin or parent tree characteristics. There are many studies with forest trees, which have indicated significant correlation for growth traits and geographic origin and these are well reviewed by Morgenstern (1996) but these inevitably rely on several years of data.

The fact that most provenances had completed flushing by early April, with only one provenance (J1) remaining partially dormant (mean score <1.0), was disappointing in that one of the major desirable phenotypic characters for walnut is late flushing. The consequence of early flushing was demonstrated in the field trials when a number of late March frosts during 1999 (Northmoor Trust, unpublished records) killed all leaves and shoots on those trees that had flushed. However, no mortality resulted and without exception, all genotypes showed excellent recovery from frost damage. The outcome was the development of new shoots from dormant buds below the damaged terminal bud, thereby creating multiple-stemmed trees. The high survival rate in the trials, although limiting any subsequent analysis, is very encouraging given the wide range of origins from which the material was collected. Future winter seasons with more extreme conditions, or later frosts in the critical flushing period in the spring, may cause additional mortality in those provenances less suited to the British climate. Indeed, in

the spring of 2000, flushing had not started by April 7th due to the cold weather, whereas by this date in 1999, the majority of trees had completed flushing. Persistence of measured phenological traits over a longer period will be another important area for future continued research.

Studies of phenology were limited to assessing flushing, partly due to constraints on time but mostly because of the difficulty of making accurate assessments of other phenological traits. In the future, analyses of leaf-fall, which were not possible within this study because the trees were confined within treeshelters, will provide a means to clarify growing season length. It was disappointing that flushing variation between provenances was mostly non-significant, thereby preventing subsequent analyses of this trait in relation to other factors, such as geographic origin. A notable variation in date of flushing in relation to altitude of origin was demonstrated by the two Turkish provenances where the mean flushing score for T2 (altitude of origin 1650 m, Table 5.1) was significantly (p=0.050) earlier than for T1 (altitude of origin 758 m) on day 85 (Figure 5.8). Such clinal variation is widely reported for many temperate tree species (Morgenstern 1996). Future phenology studies may reveal more variation as the trees grow outside the micro-climatic influence of their treeshelters. They may also provide an opportunity to assess the timing of flushing in relation to tree form, as those early flushing phenotypes are likely to have poorer form caused by repeated stem die-back at spring time. As the trees mature, it may be possible to take shoot samples in order to conduct simulated tests of frost hardiness using freezing chambers. This technique can provide accurate frost hardiness data for introduced species, such as *Alnus rubra* (Cannell *et al.* 1987) and *Nothofagus* spp. (Murray *et al.* 1986), which can be used to predict suitable planting zones within Britain based on climatological records.

Height growth in the trials was also encouraging given the small size of the trees at planting time. The variation in height increment within one site, as revealed by the Kriging method, provided an interesting insight into the importance of site selection for successful walnut growth. Provenance mean heights in relation to geographic origin (Table 5.1, p. 92) were explored but there was no obvious relationship for mean provenance height in 1999 with latitude and longitude of origin. There was a significant (p<0.001) relationship for *H*99 and altitude of origin, this being a negative trend for mean provenance height in 1999 (*H*99) against increasing altitude of origin (Figure 5.9), although the r^2 value (0.64) is not high (Table 5.21).

The assessment of phenological variation in relation to genetic variation, as revealed by isozyme analysis, was not possible due to the limited amount of genetic diversity that was

successfully resolved (Chapter 4). It is hoped that new genetic studies may be initiated in the future. The field trials will be important as a source or 'genetic bank' of material for these and similar future studies.

Table 5.21 Regression equation and analysis of variance for provenance mean height in 1999 (*H*99) and altitude of origin.

Regression equation

y* = 60.4 - 0.01*x

where *y* = *H*99 (cm) and *x* = Altitude (m)

Predictor	Coef	StDev	*T*	*p*
Constant	60.416	3.320	18.20	0.000
x	-0.013270	0.002511	-5.29	0.000

S = 6.619 r^2 = 63.6 %

Analysis of Variance

Source	*DF*	*SS*	*MS*	*F*	*p*
Regression	1	1223.7	1223.7	27.93	0.000
Error	16	701.0	43.8		
Total	17	1924.7			

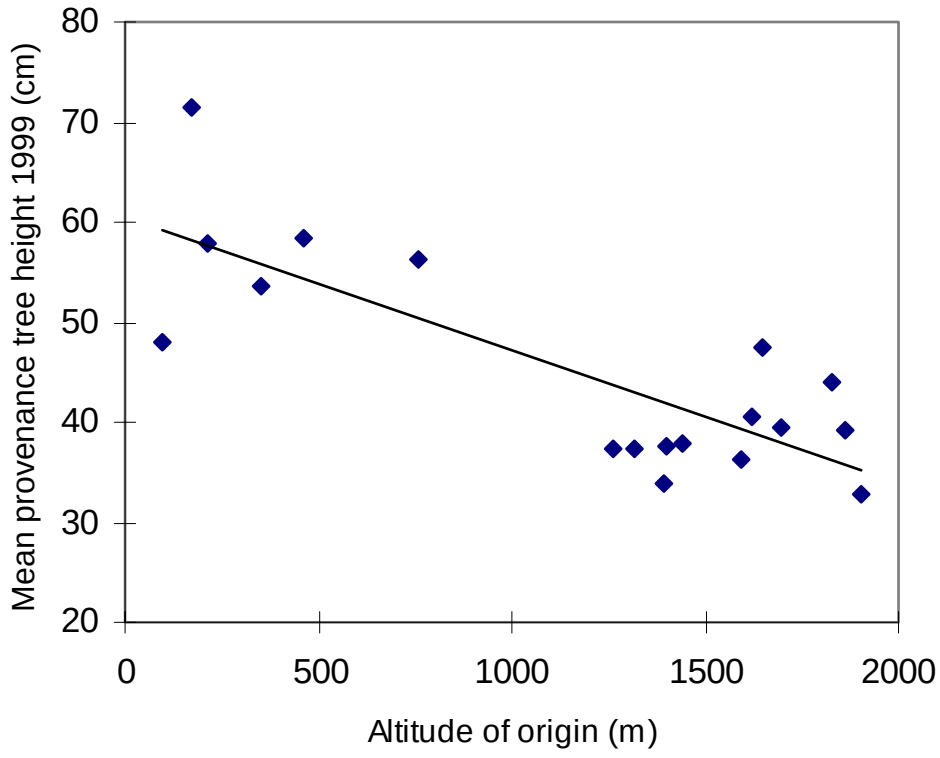


Figure 5.9 Regression plot of mean height in 1999 (*H*99) and altitude (m) of origin for 18 provenances for which altitudinal data were available (Table 5.1, p. 92).

Testing the provenances at the three field trial sites provided the means to assess genotype × environment interaction (GEI), which at this early stage was not significant. The absence of

GEI is a desirable result in that it indicates that the genotypes are equally suited to the three sites. The presence of GEI in a breeding programme often has a deleterious effect on progress towards developing superior trees because additional replication, both in space and time, is needed to allow selection for best average performance across a range of environments (Squillace 1969). If GEI is strong the tree breeder is faced with the choice of developing separate strains for each of the target environments or, if one strain is to be used across all environments, a reduction in genetic gain must be accepted as a consequence (Squillace 1969). All three sites are located within the realistic zone for walnut silviculture in southern England. If more material had been available for testing, additional sites might have been included to provide more testing environments at higher latitudes although higher mortality would inevitably have resulted. It is clear however that the Gloucestershire site, based on its environmental properties and on early growth results, will provide sufficiently testing conditions in the longer term.

At this early stage in assessing the performance of the provenances, the least promising provenances in terms of growth rates (for both height and stem diameter) and flushing, were those from Kyrgyzstan and Turkey. The Iranian (P1), Romanian (R1) and Slovakian (S1) provenances had promising growth rates although material from Spain (E1) and Tajikistan (J1) performed best both in height and diameter growth. The latter provenance was also the latest flushing among the main group of provenances (1-18). The large number of progenies within these provenances will provide an additional focus for assessments in the longer term. The French material showed good potential in terms of both growth rates and late flushing, as would be expected with these selected origins. However, assessments made over the longer term will be needed to confirm these predictions.

The collation and assessment of data after one growing season for the walnut trees in the field trials, as presented in this chapter, has provided the opportunity for clarifying the most appropriate methods of statistical analyses. These methods may be extended to all future assessments and subsequent analyses of data in this long-term field trial. Important management decisions relating to the trials will need to be taken in the future, particularly when thinning becomes necessary. Decisions will need to be undertaken with regard to the objectives of the field trials and most importantly, must address the question of whether the trials should be managed with a view to breeding improved phenotypes. This could be achieved by converting the plantations to breeding seedling orchards, although heavy thinning would be necessary. Alternatively, considerations of the genetic potential of the material may lead to the preservation of as many genotypes as possible.

Chapter 6 Conclusions and future work

COLLECTION OF GENOTYPES

The unique and substantial collection of walnut (*Juglans regia*) genotypes amassed at the start of the research programme provided an invaluable resource for the work presented here and will remain an unparalleled resource for future research in diverse fields. Based on knowledge gained through the longer-term testing of this material in the provenance/progeny trials, further collections may be conducted. New collections may be gathered from untested regions, whilst regions where existing material has shown particular promise may be revisited in order to collect more material from certain proven environments (*e.g.* particular altitudes or aspects).

SILVICULTURAL KNOWLEDGE

The dearth of silvicultural knowledge regarding the successful establishment of walnut plantations in Britain was tackled on three fronts within this study. Seed propagation and nursery bed designs were investigated with the successful production of healthy seedlings with good root systems of a desirable size for handling and planting. Gibberellins, used in breaking dormancy, provided a practical alternative to traditional stratification techniques. These techniques provided an insight into alternative methods of walnut tree production in the nursery, although more thorough research will be needed before they can be recommended to the nursery industry.

The walnut establishment trial planted at the beginning of the project was fundamental to the success of the establishment of the provenance progeny trials planted two years later. The effects of growing walnuts in different treeshelters were significant, not only on height and stem diameter growth but also on flushing and stem die-back. The taller treeshelters (120 cm), although promoting the greatest height increment, resulted in less stem diameter growth and more seriously, in the promotion of earlier flushing and greater stem die-back than smaller shelters (75 cm). Walnuts grown without treeshelters had very poor height growth in comparison to trees planted in shelters. The recommendation from this research is that shorter (75 cm) shelters should be used where possible, given the need to maintain adequate protection from browsing damage. The practice of stumping walnut trees at planting time, although promoting rapid height increment in the year following the operation, had no long term benefits in this trial, either for tree height or stem diameter growth.

The relationship between crown and stem diameters was investigated and the results directly applied in the design of the provenance/progeny trials. Using the highly significant linear

relationship (r^2 = 0.96, p = 0.000) of these two parameters it was possible to estimate the onset of crown competition, if a growth rate of 1 cm year^{-1} is presumed, at 15 to 20 years after planting at 5 m spacing. At this age, thinning will become necessary and therefore the first selections must be made in the provenance/progeny trials by 2019. A thinning regime based on the crown-stem diameter relationship was calculated and provided an indication of the potential value of growing walnut. After 60 years over 500 m^3 ha^{-1} of timber may be produced, some of which may be veneer quality timber valued up to £1000 m^3, which would amount to considerable financial return. In order to confirm the predictions made here it is hoped that measurements of crown and stem diameters will be made in the provenance/progeny trials over a long period of time, as these trials provide an ideal facility; having genotypes collected from diverse regions, planted together in pure stands. The provenance/progeny trials will also serve as an important resource in continuing to improve silvicultural knowledge, particularly by assessing gains in volume and height growth, which would provide a means to validate thinning regimes and to develop yield models.

Future silvicultural research should continue to address the techniques and timing of pruning. The potential advantages of growing walnut with companion species (Section 3.2.1) should be explored, with special regard to their potential effect on tree form, growth and provision of protection from frost through micro-climatic influence.

INTRASPECIFIC GENETIC VARIATION

The considerable time and effort devoted to isozyme analysis in the laboratory resulted in the successful generation of data for 15 systems, two of which were previously untested with *Juglans regia*. The phenolic compounds present in the tissues of *J. regia* had a strong influence on which sample extracts were effective. The low number of seeds/seedlings available for the collected genotypes precluded the adoption of the most effective sample origins, those of seed embryos or bud meristems because destructive sampling would have been necessary. Extracts from young leaves were therefore used but after early success in the laboratory, analysis of the genotypes under differing physiological and ontogenetic conditions proved less successful with the resulting production of a limited amount of data for use in population genetics studies.

Genetic diversity estimates based on data from the only consistent polymorphic locus, *Pgm*-1, indicated low levels of expected heterozygosity (H_e = 0.06), whilst a F_{ST} value of 0.05 indicated very high uniformity among populations. These genetic diversity estimates were below those calculated for the species by comparable studies, which were based on material from a narrower range. Given the wide collection of the material analysed in this study, the

use of data from a single polymorphic locus is held responsible for these low genetic diversity estimates. The data also prohibited the exploration of two further objectives within the project (Section 1.1). The goal of correlating genetic variation with provenance/progeny performance in the field trial, was an ambitious one; firstly because the genotypes had only one year in the field in which assessments of phenotypic variation could be made and secondly, the detectability of heterozygosity-fitness correlations is highly variable, not only among species but also among samples of the same species (David 1998). Furthermore, Lewontin (1984) demonstrated that there is no statistical way to compare the variation patterns between morphological traits and isozyme markers. Positive correlations between isozyme heterozygosity and fitness-related traits in plants have been recognised (Ledig 1986, Lagercrantz and Ryman 1990), although there are studies in which no correlations have been found (*e.g.* Rajora *et al.* 1991, Savolainen and Hendrick 1995). Frost hardiness or flushing was one potential phenotypic character that could be assessed within the limited time frame, and whose importance to walnut silviculture in Britain has been repeatedly stated. Hawkins *et al.* (1991) studied genetic variation and the frost hardiness of *Podocarpus totara* D. Donn, reporting that frost hardiness was positively correlated with allele frequencies at one locus of IDH. Isozyme variation however, generally provides little information on the pattern of distribution of adaptive quantitative characters, therefore important decisions which must ultimately be made on the suitability of seed transfer zones may be better placed on studies of quantitative variability (Muona 1989).

The importance of conserving intraspecific diversity for widespread species such as *Juglans regia* was discussed in Chapter 4. It was hoped that the isozyme variation data generated by this study would reveal the level and distribution of intraspecific genetic diversity and could be directly applied as an aid in decision-making directed towards the management of the walnut forests of Kyrgyzstan in promoting *in situ* gene conservation. The potential for achieving this goal, although not realised within this study, is not diminished as the collection of genotypes planted in the field trials will continue to provide a resource for future research. Furthermore, alternative quantitative markers such as microsatellites may be adopted alongside isozyme analyses in future studies. Genetic markers may also be used to monitor inbreeding and genetic diversity within a tree-breeding programme in the longer term, perhaps based on the conversion of the provenance/progeny trials to breeding seedling orchards. Genetic variability must be treated as a fundamental area for future research projects with this material, as it is one of the most important factors influencing the development of a breeding strategy (Park *et al.* 1998).

PROVENANCE AND PROGENY FIELD TRIALS

Three provenance trials were successfully established in southern England, the largest of which also doubles as a combined provenance/progeny trial. The low number of seeds, and subsequently seedlings available for planting, combined with the necessity of preserving as many individual genotypes as possible due to the requirements of the genetic diversity studies, resulted in complicated trial designs. The analysis of data from such field trial designs was made possible by the power and sophistication of modern computers and associated statistical software.

Overall, height growth of the genotypes after one growing season was encouraging given the small size of the one-year-old seedlings. The absence of genotype × environment interactions, both for height and stem diameters, indicated that at this early stage, genotypes were equally suited to the three field trial sites. After longer-term monitoring however, GEI may become significant, given the contrasting environmental conditions of the trial sites. Survival (98.9 %) was excellent across the three sites. The trees that died were significantly smaller at planting time, both in height and stem diameter, than the overall mean. Flushing assessments revealed a surprising lack of variation between both provenances and progenies. Most provenances had completed flushing by early April, which was undesirable, given the susceptibility of walnut to frost damage, and the frequency of frosts in April. Evidence from the establishment trial demonstrates that flushing of the young trees would have been strongly influenced by the presence of the treeshelters during this first growing season. The genetic gain estimate for height after one growing season was low at only 8 % although, as discussed within Chapter 5, it is likely to increase with time.

The main objective of these trials, in common with all tree improvement programmes, is to obtain a significant amount of genetic gain at a reasonable cost whilst maintaining sufficient genetic variability in the breeding populations to ensure future gain. These long-term trials will continue to generate data for many years, not only on provenance and progeny performance but may also contribute towards a greater understanding of the species silvicultural needs (*e.g.* crown canopy dynamics, volume production and height/age data). The single most important selection trait for future breeding strategies must be flushing date as this has an effect not only on survival but also on tree form. The priority in the near future will be to conduct flushing assessments when the trees have grown outside the micro-climatic influence of their treeshelters.

Juglans regia is certainly an ancient introduction to Britain but as a producer of edible nuts and, historically, this has been the main criterion for selection. Consequently, by accident rather than by design, desirable phenotypic characters for timber production have been selected against, resulting in trees of generally poor form in Britain. The initiation of a tree improvement programme, aimed at the genetic improvement of walnut for timber production, was therefore long overdue. Collections of seeds were undertaken successfully both from the species' natural and current ranges. Seedling material was generated for establishing field trials, which were planted during the final year of this research programme. Significant advances in understanding some of the silvicultural requirements of walnut were made, whilst studies of genetic variation provided a tantalising insight into the genetic diversity of the collected genotypes. The combination of these diverse strands of research, each being essential elements for a poorly studied species, contributes towards the creation of a valuable and comprehensive tree improvement programme. It is hoped that the work initiated through this research will be built upon with the ultimate aim of making walnut a profitable hardwood species for the British landowner and forester.

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Appendix I Key to provenance abbreviations

Table I.1 Provenance list

Code	Number	Origin
K1	1	Kyrgyzstan
K2	2	Kyrgyzstan
K3	3	Kyrgyzstan
K4	4	Kyrgyzstan
K5	5	Kyrgyzstan
K6	6	Kyrgyzstan
K7	7	Kyrgyzstan
K8	8	Kyrgyzstan
K9	9	Kyrgyzstan
K10	10	Kyrgyzstan
K11	11	Kyrgyzstan
E1	12	Spain
J1	13	Tadjikistan
P1	14	Iran
R1	15	Romania
S1	16	Slovakia
T1	17	Turkey
T2	18	Turkey
B1	19	Russia
B2	20	Ukraine
A1	21	Caucasus
F1	22	France
F2	23	France
F3	24	France
U1	25	United Kingdom

Table II.1 Stem straightness score and rank values (Barnes and Gibson 1986**)**

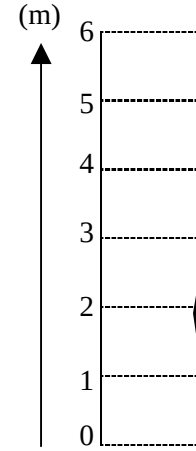
Assessment [1]	Score [2]	Rank	Assessment [1]	Score [2]	Rank
111111	21	1	221333	44	13
111122	24	2	133311	45	14
111221	26	3	133322	48	15
112211	28	4	223331	50	16
122111	30	5	333111	51	17
112222	31	6	114444	51	17
221111	32	7	333122	54	18
122122	33	8	333221	56	19
111333	33	8	224444	62	20
221122	35	9	333333	63	21
122221	35	9	144441	63	21
221221	37	10	444411	75	22
222211	39	11	444422	78	23
113331	39	11	155555	81	24
222222	42	12	555551	101	25
122333	42	12	666666	126	26

Assessment [1] figures refer to one metre sections of stem at progressively greater heights, from left to right, up to 6 metres above ground level.

Score [2] assessment values are weighted so that lower sections of stem are given greater scores, i.e. the lowest section is scored six and the highest section is scored one.

WORKED EXAMPLE FOR ASSESSMENT 223331

Each 1 m section of stem is scored according to its position within a straight length of stem: *i.e.* each 1 m section within 3 m of straight stem are each assessed as three. An assessment of 223331 therefore refers to 2 m, followed by 3 m and 1m sections respectively of straight stem. Each of the six section assessments is then given a weighted score according to its position in the stem, with lower sections given greater weighting. The sum of the weighted scores is given a ranked score, as detailed in the table above.



Stem form	Assessment	×	Weighting	=	Score
	1	×	1	=	1
	3	×	2	=	6
	3	×	3	=	9
	3	×	4	=	12
	2	×	5	=	10
	2	×	6	=	12
			Σ	**=**	**50**
			Rank	**=**	**16**

Table II.2 Kyrgyzstan provenance details

KYRGYZSTAN PROVENANCE **K1** **Region** Ak-Terek
Longitude (°E) 72.49.9 **Latitude** (°N) 41.17.5 **Altitude** (m) 1700
Aspect (°) 220 **Slope** (°) 5 **Soil Texture** Silt loam
General Description: Small plantation of pure walnut planted in the 1940s from local seed. Spaced at 8x 8 m.
Mean Basal Area: 19 m^2/ha

Tree number	**Number of seeds collected**	**Tree height** (m)	**Height to first branch** (m)	***dbh*** (cm)	**Burrs** 0=absent 1=present	**Branch Angle** [1]	**Crown diameter** (m)	**Straightness score** [2]	**Straightness rank** [2]	**Coppice** 0=not coppiced 1=coppiced	**Seed width** (mm)	**Seed length** (mm)	**Seed shape** [3]
1	5	20.4	5.0	39.0	0	2	10.8	101	25	0	24.6	33.4	6
2	5	18.5	4.0	36.0	0	1	6.9	35	9	0	28.4	35.4	6
3	5	16.8	7.1	28.5	0	0	5.7	81	24	0	25.6	34.8	6
4	5	18.3	7.0	26.0	0	1	6.6	51	17	0	28.4	29.8	1
5	5	16.2	3.6	26.8	0	1	7.8	54	18	0	28.7	33.9	1
6	5	12.9	3.5	21.2	0	1	7.6	37	10	0	29.4	34.2	1
7	5	14.7	5.2	23.0	0	1	10.3	101	25	0	30.6	37.8	1
8	5	19.6	4.0	42.3	0	1	11.2	63	21	0	27.8	30.9	1
9	5	21.2	4.4	27.7	0	1	8.7	75	22	0	29.1	31.8	1
10	5	19.0	6.9	26.2	0	1	8.3	51	17	0	30.7	36.6	1
11	5	24.0	7.0	45.2	0	1	10.8	81	24	0	31.3	35.7	1
12	5	12.3	6.3	20.4	0	1	5.6	54	18	0	32.2	35.7	1
13	5	9.3	4.8	24.9	0	0	7.8	126	26	0	31.7	39.7	6
14	5	23.2	5.2	34.2	0	1	8.4	39	11	0	29.2	38.5	4
15	5	19.2	6.0	34.8	0	2	9.1	101	25	0	27.5	38.3	6

[1] Branch angle measured from the stem: 0 = 90-60°, 1 = 60-45°, 2 ≤ 45°. [2] Straightness scores and ranks calculated according to Barnes and Gibson (1986) (Appendix Table II.1). [3] Seed shape classified according to IPGRI (1994) (Table 2.3, p.28).

Table II.2 Kyrgyzstan provenance details (continued)

KYRGYZSTAN PROVENANCE **K2** **Region** Ak-Terek
Longitude (°E) 72.50.1 **Latitude** (°N) 41.15.5 **Altitude** (m) 1390
Aspect (°) 30 **Slope** (°) 32 **Soil texture** Silt loam
General Description: Steep sided small valley containing small natural population of walnut trees. Also *Acer turkestinica* and *Crataegus* spp. and *Prunus* spp. Many walnuts were coppiced and many had burrs.
Mean Basal Area: 18 m^2/ha

Tree number	**Number of seeds collected**	**Tree height** (m)	**Height to first branch** (m)	***dbh*** (cm)	**Burrs** 0=absent 1=present	**Branch Angle** [1]	**Crown diameter** (m)	**Straightness score** [2]	**Straightness rank** [2]	**Coppice** 0=not coppiced 1=coppiced	**Seed width** (mm)	**Seed length** (mm)	**Seed shape** [3]
1	9	16.9	9.3	24.0	0	1	*	101	25	0	27.6	28.9	1
2	7	15.7	3.7	24.2	0	1	*	39	11	0	28.9	33.2	1
3	7	16.0	4.0	30.3	0	1	*	51	17	0	27.3	32.7	6
4	5	19.5	3.5	30.5	0	1	*	37	10	0	28.2	32.6	1
5	5	30.0	6.0	61.5	0	1	*	75	22	0	30.4	33.4	1
6	5	30.4	4.8	53.5	0	1	*	101	25	0	30.0	31.7	1
7	8	18.0	6.0	33.0	0	1	*	37	10	0	26.8	29.6	1
8	8	22.5	5.7	34.4	0	1	*	56	19	0	30.2	34.3	1
9	5	25.0	6.0	59.0	0	0	*	78	23	0	31.0	35.7	1
10	8	18.8	4.0	35.2	0	0	*	37	10	0	31.5	38.8	6
11	7	18.8	5.8	27.7	0	0	*	28	4	0	26.5	35.5	6
12	9	16.2	5.2	51.8	0	1	*	28	4	1	22.7	28.2	1
13	7	14.5	5.0	28.6	0	1	*	21	1	0	26.0	32.1	1
14	12	17.3	3.7	26.5	0	0	*	62	20	0	29.2	32.5	1
15	9	22.0	6.0	58.3	1	0	*	63	21	0	29.3	34.9	6
16	10	15.8	2.2	41.1	0	0	*	42	12	0	26.0	30.4	1
17	5	22.0	2.4	42.2	0	0	*	78	23	0	34.4	41.1	6

[1] Branch angle measured from the stem: 0 = 90-60°, 1 = 60-45°, 2 ≤ 45°. [2] Straightness scores and ranks calculated according to Barnes and Gibson (1986) (Appendix Table II.1). [3] Seed shape classified according to IPGRI (1994) (Table 2.3, p.28).

Table II.2 Kyrgyzstan provenance details (continued)

KYRGYZSTAN PROVENANCE **K3** **Region** Ak-Terek
Longitude (°E) 72.49.1 **Latitude** (°N) 41.17.8 **Altitude** (m) 1860
Aspect (°) 180 **Slope** (°) 26 **Soil Texture** Silt loam
General Description: Ridge top running north/south. Walnuts found on top and down east and especially west slopes. Natural forest of pure walnuts with shrubs, mostly *Crataegus* spp. at edge of walnut suitable sites.
Mean Basal Area: 23 m^2/ha

Tree number	**Number of seeds collected**	**Tree height** (m)	**Height to first branch** (m)	***dbh*** (cm)	**Burrs** 0=absent 1=present	**Branch Angle** [1]	**Crown diameter** (m)	**Straightness score** [2]	**Straightness rank** [2]	**Coppice** 0=not coppiced 1=coppiced	**Seed width** (mm)	**Seed length** (mm)	**Seed shape** [3]
1	8	15.0	2.2	46.1	0	1	13.9	39	11	0	25.8	33.5	6
2	5	19.4	8.4	33.7	0	1	9.5	62	20	0	25.6	31.9	1
3	10	23.6	7.8	31.9	0	1	*	81	24	1	25.7	33.5	1
4	7	20.3	3.0	33.4	0	1	*	54	18	1	22.4	28.8	1
5	9	20.0	4.8	46.1	0	1	12.6	28	4	0	23.6	31.8	1
6	9	15.8	5.7	34.6	0	1	10.3	44	13	0	27.0	32.3	6
7	7	31.0	8.6	29.7	0	0	7.9	101	25	0	29.4	31.3	1
8	8	19.0	3.3	33.6	0	1	9.4	37	10	0	25.7	30.6	1
9	10	13.5	4.5	27.9	0	0	10.3	75	22	0	22.9	29.1	1
10	7	18.4	3.2	40.0	0	1	12.3	37	10	0	27.3	31.1	1
11	10	13.6	4.0	27.6	0	1	*	51	17	1	21.4	24.8	1
12	10	15.0	4.5	23.3	0	1	7.2	54	18	0	20.7	27.9	1
13	9	15.0	4.5	43.2	0	1	11.6	32	7	0	26.5	35.4	1
14	8	21.0	3.8	30.1	0	1	*	32	7	1	27.7	31.8	1
15	7	12.9	4.9	19.1	0	1	6.5	39	11	0	27.0	32.2	1
16	8	15.0	3.9	30.3	0	1	8.2	21	1	0	27.1	30.4	1
17	9	15.7	4.5	25.9	0	1	7.9	75	22	0	26.3	29.6	1
18	11	22.2	7.8	28.6	0	0	*	126	26	1	25.3	30.2	1
19	10	16.6	4.9	30.5	0	0	10	39	11	0	27.1	30.6	1
20	8	16.6	4.2	29.6	0	0	8.7	39	11	0	26.3	26.9	1
21	10	18.4	4.4	37.4	0	1	11.4	31	6	0	25.5	30.5	1
22	10	17.8	4.8	43.1	0	1	11.7	51	17	0	27.8	28.8	1
23	10	15.9	2.8	24.9	0	1	9.5	21	1	0	26.2	30.1	1
24	10	13.4	5.0	25.8	0	1	8.0	51	17	0	27.3	33.4	1
25	8	15.9	5.7	27.8	0	1	8.6	39	11	0	29.5	31.4	1
26	5	17.7	5.5	29.0	0	2	8.8	63	21	0	28.8	34.1	1

[1] Branch angle measured from the stem: 0 = 90-60°, 1 = 60-45°, 2 ≤ 45°. [2] Straightness scores and ranks calculated according to Barnes and Gibson (1986) (Appendix Table II.1). [3] Seed shape classified according to IPGRI (1994) (Table 2.3, p.28).

Table II.2 Kyrgyzstan provenance details (continued)

KYRGYZSTAN PROVENANCE **K4** **Region** Sharap
Longitude (°E) 72.51.7 **Latitude** (°N) 41.16.4 **Altitude** (m) 1620
Aspect (°) 0 **Slope** (°) 22 **Soil Texture** Sandy loam
General Description: Wood pasture with open growing walnut trees with some areas of densely growing scrub of *Crataegus* spp. and *Acer* spp. Notable amount of grass on forest floor. Many walnut trees with burrs and evidence of burr removal from British expedition of the 1920's. Many coppiced forms possibly due to former burr removal.
Mean Basal Area: 19 m^2/ha

Tree number	**Number of seeds collected**	**Tree height** (m)	**Height to first branch** (m)	***dbh*** (cm)	**Burrs** 0=absent 1=present	**Branch Angle** [1]	**Crown diameter** (m)	**Straightness score** [2]	**Straightness rank** [2]	**Coppice** 0=not coppiced 1=coppiced	**Seed width** (mm)	**Seed length** (mm)	**Seed shape** [3]
1	10	19.2	6.0	46.0	0	1	9.9	51	17	0	25.0	30.1	1
2	10	23.6	2.6	95.5	1	1	16.0	39	11	1	29.9	37.5	1
3	8	15.7	7.5	40.5	1	0	10.8	62	20	0	29.3	34.5	6
4	10	26.0	3.4	88.5	1	1	13.7	56	19	0	28.2	28.9	1
5	8	25.2	8.7	83.2	0	0	19.2	63	21	0	27.1	30.6	1
6	5	16.8	8.5	26.1	0	2	7.2	126	26	0	26.5	27.3	1
7	10	15.2	2.7	34.3	0	0	8.8	48	15	0	28.8	32.1	1
8	8	15.0	1.8	62.9	1	1	17.1	28	4	0	29.0	35.6	6
9	11	24.0	1.7	93.1	0	1	*	21	1	1	27.5	30.4	1
10	11	23.5	6.4	44.0	1	0	12.8	42	12	0	25.1	32.2	6
11	6	23.3	12.0	30.5	0	0	*	37	10	1	31.9	35.7	1
12	6	26.6	1.5	83.4	1	1	12.0	101	25	0	31.2	30.7	1
13	11	22.2	2.5	26.7	0	1	9.8	21	1	0	27.2	30.4	1
14	9	21.3	10.4	41.6	0	1	10.1	44	13	0	30.0	32.4	1
15	9	16.5	7.5	23.0	0	0	9.9	78	23	0	28.3	31.8	1
16	10	24.7	7.8	32.9	0	1	*	28	4	1	25.5	25.2	1
17	10	28.1	5.5	99.5	1	1	*	50	16	1	27.5	30.7	1
18	10	13.2	4.5	24.6	0	1	7.0	126	26	0	28.1	32.2	1
19	10	25.5	7.5	34.1	1	1	*	35	9	1	30.7	34.6	1
20	10	16.5	5.8	23.4	0	0	9.1	101	25	0	26.8	32.7	6
21	10	24.8	5.4	67.5	1	0	13.0	126	26	1	33.9	36.6	1
22	10	23.3	5.7	88.1	0	1	*	63	21	0	26.6	30.9	1
23	10	13.0	7.8	17.1	0	0	5.3	51	17	0	29.4	32.9	1
24	10	21.0	3.0	61.5	1	1	*	39	11	0	28.7	32.2	6
25	10	23.3	5.8	65.3	0	1	*	62	20	1	27.1	30.8	6
26	10	23.9	4.3	102.0	1	0	16.8	78	23	0	28.3	30.9	1
27	10	16.9	3.0	47.3	0	1	10.5	56	19	0	30.7	32.7	1

[1] Branch angle measured from the stem: 0 = 90-60°, 1 = 60-45°, 2 ≤ 45°. [2] Straightness scores and ranks calculated according to Barnes and Gibson (1986) (Appendix Table II.1). [3] Seed shape classified according to IPGRI (1994) (Table 2.3, p.28).

Table II.2 Kyrgyzstan provenance details (continued)

KYRGYZSTAN PROVENANCE **K5** **Region** Yaradar (near Arslanbob)
Longitude (°E) 72.59.0 **Latitude** (°N) 41.19.2 **Altitude** (m) 1260
Aspect (°) 180 **Slope** (°) 10 **Soil Texture** Silt loam
General Description: Low seed production in this area. Tree forms not phenotypically superior but many very old trees over 200 years. One other canopy tree species noted in the forest – *Fraxinus* spp. Grass vegetation under canopy. Indications of burr removal but not recent.
Mean Basal Area: 20 m^2/ha

Tree number	**Number of seeds collected**	**Tree height** (m)	**Height to first branch** (m)	***dbh*** (cm)	**Burrs** 0=absent 1=present	**Branch Angle** [1]	**Crown diameter** (m)	**Straightness score** [2]	**Straightness rank** [2]	**Coppice** 0=not coppiced 1=coppiced	**Seed width** (mm)	**Seed length** (mm)	**Seed shape** [3]
1	7	22.8	9.3	39.2	0	1	*	126	26	1	31.1	34.4	1
2	6	17.3	7.5	28.8	0	1	*	51	17	1	25.9	30.7	1
3	8	29.2	3.5	84.0	1	1	*	75	22	1	31.7	33.1	1
4	6	10.8	3.8	22.2	0	1	*	39	11	1	26.7	29.4	1
5	8	15.3	12.4	25.5	0	1	*	28	4	1	28.9	34.8	1
6	10	18.1	4.8	85.2	1	1	*	51	17	0	27.1	34.4	1
7	7	16.4	6.6	55.3	1	1	*	32	7	0	27.2	32.6	1
8	10	20.3	4.1	58.3	0	1	*	21	1	1	27.8	31.0	1
9	8	13.1	5.4	31.6	0	1	*	32	7	0	29.3	32.3	1
10	10	18.0	3.0	44.5	1	1	*	51	17	0	31.4	39.1	1
11	7	22.2	3.9	62.6	0	1	*	75	22	0	26.3	29.2	1
12	10	24.7	5.2	104.5	1	1	*	75	22	0	27.9	34.4	1
13	6	14.2	4.0	29.8	0	1	*	56	19	1	28.8	37.1	3
14	10	22.7	6.0	65.7	1	1	*	126	26	0	25.7	29.8	1
15	5	31.3	2.0	116.0	1	1	*	101	25	0	29.1	30.9	1
16	10	23.9	4.5	54.0	0	1	*	39	11	1	32.7	34.8	1
17	6	19.5	7.0	19.9	0	0	*	30	5	0	31.5	30.5	1
18	10	22.5	1.9	101.8	0	1	*	78	23	0	28.6	31.7	1
19	10	25.4	4.2	94.3	1	1	*	78	23	0	25.0	30.5	1
20	10	20.0	3.9	93.5	0	1	*	56	19	1	28.5	29.1	1

[1] Branch angle measured from the stem: 0 = 90-60°, 1 = 60-45°, 2 ≤ 45°. [2] Straightness scores and ranks calculated according to Barnes and Gibson (1986) (Appendix Table II.1). [3] Seed shape classified according to IPGRI (1994) (Table 2.3, p.28).

Table II.2 Kyrgyzstan provenance details (continued)

KYRGYZSTAN PROVENANCE **K6** **Region** Shaidan
Longitude (°E) 72.47.7 **Latitude** (°N) 41.16.8 **Altitude** (m) 1590
Aspect (°) 60 **Slope** (°) 20 **Soil Texture** Clay loam
General Description: Trees younger than in some of the other sampled sites. On steeper slopes other species found: *Acer turkestanica* and *Crataegus turkestanica*. Grassy vegetation and heavy grazing present.
Mean Basal Area: 23 m^2/ha

Tree number	**Number of seeds collected**	**Tree height** (m)	**Height to first branch** (m)	***dbh*** (cm)	**Burrs** 0=absent 1=present	**Branch Angle** [1]	**Crown diameter** (m)	**Straightness score** [2]	**Straightness rank** [2]	**Coppice** 0=not coppiced 1=coppiced	**Seed width** (mm)	**Seed length** (mm)	**Seed shape** [3]
1	10	17.6	3.2	39.5	0	0	*	35	9	0	29.3	32.6	1
2	10	19.2	3.0	67.2	1	0	*	39	11	1	27.6	30.7	1
3	10	17.6	3.3	27.9	0	1	*	35	9	0	28.2	33.0	1
4	7	17.7	5.7	29.4	0	1	*	51	17	0	34.6	36.3	1
5	10	18.8	6.7	30.1	0	0	*	126	26	0	24.0	27.4	1
6	10	24.0	7.5	52.1	1	1	*	126	26	1	29.1	29.7	1
7	10	24.7	3.1	55.6	0	2	*	51	17	0	23.4	28.4	1
8	8	27.6	16.4	30.8	0	1	*	126	26	0	27.1	30.3	1
9	9	15.8	3.0	24.3	0	1	*	56	19	0	27.9	33.6	1
10	7	19.9	7.2	28.1	0	2	*	101	25	1	26.6	32.2	1
11	10	23.3	8.6	23.6	0	2	*	54	18	0	27.6	29.8	1
12	10	28.4	9.4	32.6	0	1	*	101	25	0	28.7	37.3	1
13	10	20.9	7.4	42.0	0	2	*	56	19	0	27.9	37.7	6
14	10	19.5	7.5	33.7	0	1	*	37	10	0	25.3	35.4	6
15	10	21.0	9.0	32.9	0	0	*	126	26	0	26.0	27.3	1
16	10	19.2	7.5	45.5	1	1	*	126	26	0	25.2	30.2	1
17	10	6.7	1.8	12.8	0	0	*	39	11	0	27.7	32.0	1
18	10	17.3	6.0	44.2	0	1	*	101	25	1	24.3	28.6	1
19	10	15.0	6.7	30.1	0	1	*	35	9	0	26.0	31.8	1
20	7	14.3	7.5	26.9	0	1	*	101	25	0	28.0	30.8	1
21	10	23.3	6.0	39.5	0	1	*	24	2	1	25.6	31.6	1
22	10	19.5	6.0	39.6	0	0	*	126	26	0	24.2	30.8	1
23	10	10.5	4.1	31.4	0	1	*	78	23	0	30.0	39.6	6
24	10	11.8	6.6	25.0	0	1	*	35	9	0	27.1	31.5	1
25	10	15.6	3.2	30.4	0	0	*	39	1	0	27.7	33.9	1
26	10	15.8	8.2	32.6	0	0	*	51	17	0	26.0	28.7	1

[1] Branch angle measured from the stem: 0 = 90-60°, 1 = 60-45°, 2 ≤ 45°. [2] Straightness scores and ranks calculated according to Barnes and Gibson (1986) (Appendix Table II.1). [3] Seed shape classified according to IPGRI (1994) (Table 2.3, p.28).

Table II.2 Kyrgyzstan provenance details (continued)

KYRGYZSTAN PROVENANCE **K7** **Region** Kyzyl-Ungur (Red Cave)
Longitude (°E) 73.05.7 **Latitude** (°N) 41.23.1 **Altitude** (m) 1400
Aspect (°) 330 **Slope** (°) 27 **Soil Texture** Sandy loam
General Description: Long grassy vegetation and natural walnut regeneration on steeper slopes (30^0 +). *Juniperus turkestanica* on ridge tops above limit of walnut. Wild mint and tormentil present. Low seed yields. Sampling from tree 6 onwards took place in another valley approximately 1 km away to the east.
Mean Basal Area: 17 m^2/ha

Tree number	**Number of seeds collected**	**Tree height** (m)	**Height to first branch** (m)	***dbh*** (cm)	**Burrs** 0=absent 1=present	**Branch Angle** [1]	**Crown diameter** (m)	**Straightness score** [2]	**Straightness rank** [2]	**Coppice** 0=not coppiced 1=coppiced	**Seed width** (mm)	**Seed length** (mm)	**Seed shape** [3]
1	10	22.7	7.5	50.4	0	1	*	126	26	1	27.9	35.0	1
2	10	27.2	2.5	91.9	1	0	15.1	126	26	0	28.3	33.7	1
3	7	17.3	7.5	38.9	0	1	*	30	5	0	23.8	29.4	1
4	9	24.7	5.8	63.4	1	1	*	75	22	0	26.1	29.4	1
5	10	20.4	6.0	58.2	0	0	*	28	4	0	32.7	32.4	1
6	10	21.8	6.5	54.3	0	2	*	75	22	0	27.3	34.4	1
7	10	12.9	2.5	26.5	0	1	*	42	12	0	32.8	35.6	1
8	10	16.5	3.0	24.7	0	1	*	48	15	0	31.3	47.6	7
9	17	18.0	6.5	25.6	0	0	*	81	24	0	28.7	33.7	1
10	18	26.2	8.7	36.2	0	1	*	33	8	1	31.6	33.7	1
11	19	25.4	4.5	73.7	0	1	*	50	16	0	27.3	30.0	3
12	12	21.7	9.0	58.1	0	2	*	33	8	1	30.8	30.8	1
13	12	22.1	1.9	56.5	0	2	*	21	1	1	27.9	29.5	1
14	12	19.7	4.6	39.0	0	1	*	32	7	0	27.3	32.5	1
15	11	29.9	8.2	47.3	1	1	*	78	23	1	27.2	33.5	1
16	14	17.3	7.5	38.8	0	0	*	42	12	1	26.4	28.1	1
17	12	30.3	7.4	70.5	0	1	*	35	9	0	25.6	31.7	1

[1] Branch angle measured from the stem: 0 = 90-60°, 1 = 60-45°, 2 ≤ 45°. [2] Straightness scores and ranks calculated according to Barnes and Gibson (1986) (Appendix Table II.1). [3] Seed shape classified according to IPGRI (1994) (Table 2.3, p.28).

Table II.2 Kyrgyzstan provenance details (continued)

KYRGYZSTAN PROVENANCE **K8** **Region** Katar-Yangak
Longitude (°E) 72.50.8 **Latitude** (°N) 41.18.7 **Altitude** (m) 1900
Aspect (°) 160 **Slope** (°) 10 **Soil Texture** Silt loam
General Description: Ravine running downslope at 160^0 aspect, about 15 m deep. Walnuts growing on steep sided (40^0) side slopes. Many thin shelled nuts noticed in the area (Tree 9 very thin shelled).
Mean Basal Area: 23 m^2/ha

Tree number	**Number of seeds collected**	**Tree height** (m)	**Height to first branch** (m)	***dbh*** (cm)	**Burrs** 0=absent 1=present	**Branch Angle** [1]	**Crown diameter** (m)	**Straightness score** [2]	**Straightness rank** [2]	**Coppice** 0=not coppiced 1=coppiced	**Seed width** (mm)	**Seed length** (mm)	**Seed shape** [3]
1	10	10.2	4.3	18.7	0	0	*	45	14	0	28.0	34.1	1
2	10	13.2	5.9	30.0	0	1	*	39	11	0	24.5	27.6	1
3	10	26.7	7.6	50.2	1	1	*	75	22	0	25.8	28.2	1
4	8	26.7	3.4	58.3	1	0	*	44	13	0	32.0	35.9	1
5	11	21.7	6.8	72.0	1	1	*	63	21	0	27.0	29.9	1
6	10	22.5	10.8	35.2	0	1	*	54	18	0	26.8	28.1	1
7	10	25.8	3.0	107.3	1	1	*	56	19	0	27.7	33.2	1
8	10	21.0	1.0	43.3	0	1	*	21	1	0	28.0	32.7	6
9	16	15.0	5.0	23.8	0	1	*	21	1	0	22.3	24.9	1
10	10	15.7	4.5	37.3	0	0	*	44	13	0	30.7	28.5	1
11	10	18.7	5.2	43.4	0	1	*	48	15	1	28.2	33.7	1
12	10	20.3	4.5	43.8	0	1	*	54	18	0	24.8	27.8	1
13	10	21.0	2.0	40.5	0	1	*	39	11	0	27.6	33.1	1
14	10	14.2	4.4	59.9	1	1	*	78	23	0	24.9	27.0	1
15	12	19.5	6.8	35.6	0	1	*	39	11	0	24.6	29.7	1
16	11	19.5	9.8	35.1	0	1	*	62	20	0	24.7	27.7	1
17	11	18.0	3.6	23.5	0	1	*	81	24	0	25.5	26.2	1
18	11	15.6	8.3	23.4	0	1	*	21	1	0	26.2	25.9	1
19	10	20.1	9.6	30.7	0	0	*	42	12	0	29.1	30.4	1
20	10	20.8	4.9	36.5	0	1	*	78	23	0	28.8	29.9	1
21	10	18.7	6.5	29.0	0	1	*	81	24	0	25.5	27.5	1
22	10	21.0	4.0	78.7	1	1	*	39	11	1	24.8	29.6	1
23	10	19.8	4.6	45.9	0	2	*	63	21	0	28.9	30.2	1
24	10	14.1	5.2	30.9	0	2	*	51	17	0	24.0	27.2	1
25	10	15.6	4.0	44.9	0	2	*	30	5	0	23.4	25.6	1

[1] Branch angle measured from the stem: 0 = 90-60°, 1 = 60-45°, 2 ≤ 45°. [2] Straightness scores and ranks calculated according to Barnes and Gibson (1986) (Appendix Table II.1). [3] Seed shape classified according to IPGRI (1994) (Table 2.3, p.28).

Table II.2 Kyrgyzstan provenance details (continued)

KYRGYZSTAN PROVENANCE **K9** **Region** Kyok-Sarai
Longitude (°E) 72.53.1 **Latitude** (°N) 41.18.0 **Altitude** (m) 1830
Aspect (°) 40 **Slope** (°) 8 **Soil Texture** Silt loam
General Description: Grassy vegetation and heavily grazed. Wood pasture on flatter slopes.
Mean Basal Area: 16 m^2/ha

Tree number	**Number of seeds collected**	**Tree height** (m)	**Height to first branch** (m)	***dbh*** (cm)	**Burrs** 0=absent 1=present	**Branch Angle** [1]	**Crown diameter** (m)	**Straightness score** [2]	**Straightness rank** [2]	**Coppice** 0=not coppiced 1=coppiced	**Seed width** (mm)	**Seed length** (mm)	**Seed shape** [3]
1	10	23.7	6.8	41.2	0	0	8.6	21	1	1	29.8	33.4	1
2	10	15.9	2.5	58.2	1	1	*	21	1	1	26.3	32.4	1
3	10	9.1	2.5	16.7	0	2	6.2	21	1	0	29.9	31.5	1
4	10	20.2	4.0	82.8	1	1	16.3	126	26	0	29.0	31.3	1
5	10	19.9	2.8	76.4	1	1	*	30	5	1	27.5	34.2	1
6	10	11.7	4.5	24.2	0	1	7.3	32	7	0	29.9	34.9	1
7	10	22.5	9.7	66.1	1	2	12.2	75	22	0	29.4	33.1	1
8	10	18.1	4.4	60.5	0	1	14.6	35	9	0	30.8	36.4	6
9	10	19.8	5.8	23.8	1	0	*	39	11	1	31.2	33.0	1
10	10	15.0	5.3	31.0	0	0	*	39	11	1	32.0	29.9	1
11	10	15.3	4.7	22.2	0	1	7	44	13	1	29.9	36.8	6
12	10	18.9	5.2	54.8	1	1	*	56	19	1	33.5	34.7	1
13	10	29.9	4.1	87.4	1	1	*	54	18	1	28.1	30.8	1
14	10	28.0	8.2	60.4	1	1	*	50	16	1	26.1	26.6	1
15	10	22.2	4.1	59.6	1	1	*	39	11	1	30.6	34.2	6
16	10	18.7	5.2	41.5	0	1	*	56	19	1	33.8	35.8	1
17	10	21.2	3.7	65.8	0	1	16.9	78	23	0	27.7	34.9	7
18	10	21.5	3.4	70.5	1	1	13.9	75	22	0	30.0	33.5	1
19	10	17.1	5.7	57.8	1	1	*	39	11	1	28.7	34.0	1
20	10	16.5	5.3	32.3	0	1	*	42	12	0	28.1	27.2	1
21	10	12.6	3.3	23.7	0	1	*	21	1	0	30.8	38.0	6
22	9	17.1	4.8	70.6	1	0	*	62	20	0	28.1	37.0	6
23	10	17.6	3.0	58.3	0	0	11.1	51	17	1	29.0	32.4	1
24	10	10.2	4.2	19.5	0	1	*	63	21	1	31.2	31.4	1
25	10	12.3	2.9	25.3	0	1	8.6	63	21	0	32.9	38.1	6
26	10	18.1	6.2	56.4	0	1	*	26	3	1	27.4	27.8	1

[1] Branch angle measured from the stem: 0 = 90-60°, 1 = 60-45°, 2 ≤ 45°. [2] Straightness scores and ranks calculated according to Barnes and Gibson (1986) (Appendix Table II.1). [3] Seed shape classified according to IPGRI (1994) (Table 2.3, p.28).

Table II.2 Kyrgyzstan provenance details (continued)

KYRGYZSTAN PROVENANCE **K10** **Region** Kyr-Sai (Sary-Chalek Biosphere Reserve)
Longitude (°E) 71.57.4 **Latitude** (°N) 41.50.6 **Altitude** (m) 1320
Aspect (°) 60 **Slope** (°) 9 **Soil Texture** Sandy loam
General Description: Sampled from one side of the valley, tree 1 at highest elevation. Many good phenotypes and many very old trees. Large boulders in some places. Grassy vegetation and scrub present. Tree 7 probably over 300 years old. Tree 16 best phenotype observed at any location.
Mean Basal Area: 13 m^2/ha

Tree number	**Number of seeds collected**	**Tree height** (m)	**Height to first branch** (m)	***dbh*** (cm)	**Burrs** 0=absent 1=present	**Branch Angle** [1]	**Crown diameter** (m)	**Straightness score** [2]	**Straightness rank** [2]	**Coppice** 0=not coppiced 1=coppiced	**Seed width** (mm)	**Seed length** (mm)	**Seed shape** [3]
1	10	24.0	7.2	37.1	0	1	*	126	26	0	22.7	25.1	1
2	10	21.8	6.0	50.3	1	0	13.1	63	21	0	22.4	28.7	7
3	10	28.8	9.7	86.8	1	0	12.7	78	23	1	27.4	31.8	1
4	10	18.6	6.9	36.3	0	0	*	42	12	1	27.1	32.3	6
5	10	20.7	6.9	35.1	0	0	*	63	21	0	27.8	28.9	1
6	10	19.4	6.1	36.3	0	1	*	51	17	0	27.1	31.1	1
7	9	34.0	6.2	128.3	1	1	14.8	78	23	0	26.6	32.2	6
8	10	14.5	3.8	39.0	1	1	*	62	20	0	28.6	32.8	1
9	10	22.4	10.9	42.9	0	1	*	126	26	0	32.0	35.4	6
10	10	16.2	5.0	42.2	0	1	*	56	19	0	27.5	27.9	1
11	10	33.0	9.9	115.5	1	1	15.2	51	17	0	28.9	30.8	1
12	10	18.8	10.5	24.7	0	1	*	42	12	0	29.4	35.8	1
13	10	17.3	10.5	31.9	0	0	*	51	17	0	26.1	29.6	1
14	10	22.5	5.8	114.9	1	0	*	39	11	1	31.0	35.0	1
15	10	18.3	5.4	43.5	0	0	*	101	25	0	32.6	35.7	1
16	10	26.0	17.7	36.7	0	0	*	126	26	0	25.7	31.0	7
17	10	17.4	9.7	23.4	0	1	7.5	54	18	0	26.7	31.8	1
18	10	17.0	6.4	32.8	0	1	*	62	20	0	26.4	28.1	1
19	10	16.8	8.0	17.0	0	1	*	63	21	0	38.2	32.2	1
20	10	23.7	7.2	33.3	0	2	*	31	6	0	28.7	30.3	1
21	10	16.0	5.8	36.8	0	0	*	39	11	0	23.0	30.3	1
22	10	11.3	5.8	19.5	0	1	*	63	21	0	31.7	33.6	1
23	10	23.3	8.3	37.4	0	1	*	101	25	0	31.1	33.2	1
24	10	11.4	4.2	22.2	0	1	*	75	22	0	38.6	32.0	1
25	10	22.9	10.9	35.8	0	1	*	35	9	0	27.2	31.3	1
26	10	15.7	6.3	29.5	0	1	*	78	23	0	27.9	29.0	1
27	10	23.3	8.7	38.1	0	1	*	44	13	0	29.7	33.1	6

[1] Branch angle measured from the stem: 0 = 90-60°, 1 = 60-45°, 2 ≤ 45°. [2] Straightness scores and ranks calculated according to Barnes and Gibson (1986) (Appendix Table II.1). [3] Seed shape classified according to IPGRI (1994) (Table 2.3, p.28).

Table II.2 Kyrgyzstan provenance details (continued)

KYRGYZSTAN PROVENANCE **K11** **Region** Ters-Kolt (Sary-Chalek Biosphere Reserve)
Longitude (°E) 71.56.6 **Latitude** (°N) 41.49.5 **Altitude** (m) 1440
Aspect (°) 0 **Slope** (°) 10 **Soil Texture** Sandy loam
General Description: Sheltered side valley with steep sides. Some heavy grazing except on steeper slopes. Wide range of age classes.
Mean Basal Area: 13 m^2/ha

Tree number	**Number of seeds collected**	**Tree height** (m)	**Height to first branch** (m)	***dbh*** (cm)	**Burrs** 0=absent 1=present	**Branch Angle** [1]	**Crown diameter** (m)	**Straightness score** [2]	**Straightness rank** [2]	**Coppice** 0=not coppiced 1=coppiced	**Seed width** (mm)	**Seed length** (mm)	**Seed shape** [3]
1	10	18.0	6.8	39.4	0	0	*	81	24	0	28.1	31.8	1
2	10	18.0	7.5	33.6	0	0	*	126	26	0	30.0	36.5	7
3	10	12.3	7.2	18.6	0	0	*	42	12	0	28.5	34.1	6
4	10	24.7	7.2	112.7	1	0	13.7	78	23	1	28.1	29.0	1
5	10	12.6	5.8	22.3	0	1	*	63	21	0	29.7	29.5	1
6	10	16.6	5.7	23.3	0	0	*	75	22	0	33.2	33.3	1
7	10	13.5	5.9	20.4	0	0	*	126	26	1	25.9	32.2	7
8	10	34.0	9.3	100.0	1	1	*	62	20	0	30.6	32.0	1
9	10	30.4	11.4	79.3	1	1	*	63	21	0	29.6	31.4	1
10	10	27.6	9.6	72.5	0	1	*	35	9	1	28.3	31.5	1
11	10	10.1	5.2	14.0	0	0	*	28	4	0	28.5	30.9	1
12	10	13.5	4.9	28.0	0	0	*	101	25	1	25.8	28.3	4
13	10	28.6	12.1	55.6	0	1	*	78	23	0	27.1	34.8	7
14	10	15.7	7.1	23.0	0	0	*	78	23	1	25.7	27.7	1
15	10	18.0	5.4	19.4	0	1	*	126	26	1	30.2	34.2	1
16	10	10.2	4.0	18.2	0	1	*	75	22	1	29.1	33.8	6
17	10	21.4	14.4	32.3	0	0	*	63	21	0	30.8	33.5	1
18	10	14.6	5.3	20.1	0	1	*	51	17	0	32.5	36.7	4
19	10	14.6	7.2	30.8	0	1	*	44	13	0	26.2	30.7	1
20	10	17.4	9.4	36.7	0	0	*	39	11	1	29.6	31.7	1
21	10	15.2	8.8	24.7	0	0	*	78	23	0	29.5	31.1	1
22	10	28.8	8.2	112.0	1	0	*	63	21	0	26.8	29.9	1
23	10	19.2	4.5	46.0	0	0	*	78	23	0	29.0	30.9	1
24	10	27.0	7.0	89.8	1	0	14.8	63	21	0	28.0	32.5	1
25	10	19.5	7.1	28.5	0	1	*	81	24	0	31.1	34.5	1
26	10	12.8	6.1	21.8	0	0	*	33	8	1	29.7	34.4	1
27	10	19.1	4.7	60.5	1	0	*	78	23	0	24.4	32.3	7

[1] Branch angle measured from the stem: 0 = 90-60°, 1 = 60-45°, 2 ≤ 45°. [2] Straightness scores and ranks calculated according to Barnes and Gibson (1986) (Appendix Table II.1). [3] Seed shape classified according to IPGRI (1994) (Table 2.3, p.28).

Table II.3 Location and altitude of the parent trees in the Romanian provenance (R1).

Progeny	Location (town/village)	County	Altitude (metres a.s.l.)
265	Buzau	Buzau	480
266	Giurgiu	Giurgiu	60
267	Fagaras	Brasov	570
268	Fagaras	Brasov	590
269	Rotbav	Brasov	480
270	Ploiesti	Prahova	430
271	Ploiesti	Prahova	410
272	Aricesti-Zeletin	Prahova	650
273	Aricesti-Zeletin	Prahova	630
274	Galati	Galati	80
275	Tecuci	Galati	60
276	Maneciu-Paminteni	Prahova	605
277	Maneciu-Ungureni	Prahova	620
278	Pojorta	Brasov	650
279	Pojorta	Brasov	640
280	Valeni (Cireasa)	Dimbovita	650
281	Risnov	Brasov	620
282	Remeti	Maramures	630
283	Piatra	Maramures	640
284	Motaieni	Dimbovita	325
285	Motaieni	Dimbovita	325
286	Motaieni	Dimbovita	325
287	Brasov	Brasov	520
288	Panciu	Vrancea	420
289	Cimpuri	Vrancea	410
290	Bacau	Bacau	460
291	Romos	Hunedoara	620

Table II.4 Locations and altitudes of the parent trees in the Slovakian provenance (S1), and summary temperature and precipitation data (Sojak[1], pers. comm.).

Progeny	Location	Latitude (° North)	Longitude (° East)	Altitude (metres a.s.l.)	Mean annual rainfall (mm)	Mean annual temperature (°C)
292	Kolárovo	47.75	18.00	110	546	10.0
293	Kolárovo	47.75	18.00	110	546	10.0
294	Kolárovo	47.75	18.00	110	546	10.0
295	Kolárovo	47.75	18.00	110	546	10.0
296	Nová Dedina	48.25	18.50	220	561	9.7
297	Nová Dedina	48.25	18.50	220	561	9.7
298	Nová Dedina	48.25	18.50	220	561	9.7
299	Nová Dedina	48.25	18.50	220	561	9.7
300	Nová Dedina	48.25	18.50	220	561	9.7
301	Nová Dedina	48.25	18.50	220	561	9.7
302	Nová Dedina	48.25	18.50	220	561	9.7
303	Nová Dedina	48.25	18.50	220	561	9.7
304	Nová Dedina	48.25	18.50	220	561	9.7
305	Kovácová	48.60	19.00	295	715	7.9
306	Kovácová	48.60	19.00	295	715	7.9
307	Badín	48.60	19.00	320	715	7.9
308	Badín	48.60	19.00	320	715	7.9
309	Badín	48.60	19.00	320	715	7.9
310	Gregorová Vieska	48.40	19.75	190	620	8.6
311	Poltár	48.50	19.75	200	620	8.6
312	Kalinovo	48.50	19.75	200	620	8.6
313	Lucenec	48.40	19.75	200	620	8.6
314	Lucenec	48.40	19.75	200	620	8.6
315	Lucenec	48.40	19.75	200	620	8.6
316	Lucenec	48.40	19.75	200	620	8.6
317	Lucenec	48.40	19.75	200	620	8.6
318	Lucenec	48.40	19.75	200	620	8.6
319	Lucenec	48.40	19.75	200	620	8.6
320	Lucenec	48.40	19.75	200	620	8.6
321	Lucenec	48.40	19.75	200	620	8.6
322	Lucenec	48.40	19.75	200	620	8.6
323	Lucenec	48.40	19.75	200	620	8.6
324	Lucenec	48.40	19.75	200	620	8.6
325	Málinec	48.5	19.75	350	650	8.0
			MEAN	**215**	**611**	**8.9**

[1] Dr Dusan Sojak, Forest Research Institute, Slovakia.

Table II.5 Location and details of parent trees within Turkish provenance T1.

Progeny	Area within Trabzon region	Parent tree height (m)	Parent tree *dbh* (cm)	Estimated age of parent tree (years)	Altitude (metres a.s.l.)
326	Esiroglu-Maaka	18	42	28	200
327	Esiroglu-Maaka	19	42	42	210
328	Esiroglu-Maaka	47	51	50	260
329	Esiroglu-Maaka	22	43	30	360
330	Esiroglu-Maaka	16	51	50	440
331	Esiroglu-Maaka	20	34	20	540
332	Esiroglu-Maaka	20	30	25	620
333	Esiroglu-Maaka	20	50	30	720
334	Hamsikoy-Maaka	15	27	15	970
335	Hamsikoy-Maaka	20	36	15	950
336	Hamsikoy-Maaka	25	80	50	900
337	Hamsikoy-Maaka	12	21	30	900
338	Hamsikoy-Maaka	10	26	20	900
339	Hamsikoy-Maaka	20	34	20	900
340	Hamsikoy-Maaka	30	70	50	1500
341	Hamsikoy-Maaka	20	35	22	1500
342	Hamsikoy-Maaka	40	54	40	1500
343	Hamsikoy-Maaka	40	56	35	900
344	Hamsikoy-Maaka	12	20	15	900
345	Meryemana-Maaka	30	48	30	1200
346	Meryemana-Maaka	30	41	25	1200
347	Meryemana-Maaka	30	35	25	1200
348	Ugurlu Koyu	15	30	20	400
349	Of-Tekoba	25	35	40	300
350	Of-Tekoba	20	35	25	350
351	Of-Tekoba	15	20	20	350
	Mean	**23**	**40**	**30**	**776**

Table III.1 Walnut establishment trial; plot summary data.

Block	Plot	Population	Treeshelter	Stump	H96	D96	H97	IH97	H98	D98	IH98	ID96-98	H99	D99	IH99	ID99	IH96-99	ID96-99	N96	N99
1	1	2	120	0	73.8	18.4	128.3	54.6	144.8	21.7	16.6	3.3	181.7	30.2	36.9	8.5	108.0	11.8	16	16
1	2	1	75	1	10.0	14.2	100.1	90.1	124.4	24.2	24.3	10.0	154.0	33.5	29.6	9.3	144.0	19.3	16	16
1	3	2	120	1	10.0	17.4	115.0	105.0	133.6	21.1	18.6	3.7	173.3	28.3	39.7	7.2	163.3	10.9	16	16
1	4	2	75	0	83.4	19.0	94.7	11.2	117.4	25.8	22.7	6.8	160.1	34.4	42.7	8.7	76.6	15.4	16	16
1	5	1	0	0	37.5	17.4	47.6	10.1	60.0	18.4	12.4	1.0	95.3	25.8	35.3	7.5	57.8	8.4	16	16
1	6	1	120	0	43.8	20.4	141.3	97.5	134.0	18.8	-7.3	-1.7	163.4	24.6	29.4	5.8	119.6	4.1	16	16
1	7	2	0	1	10.0	18.7	37.6	27.6	46.3	13.0	8.7	-5.7	81.4	21.5	35.1	8.5	71.4	2.8	16	16
1	8	1	120	1	10.0	12.3	139.6	129.6	138.1	18.6	-1.5	6.3	165.1	23.9	27.0	5.3	155.1	11.6	16	16
1	9	2	0	0	80.6	16.9	54.6	-26.0	58.7	14.6	2.4	-2.2	76.3	19.9	17.6	5.2	-4.4	3.0	16	16
1	10	1	0	1	10.0	14.6	41.8	31.8	50.7	11.7	8.9	-2.9	73.9	19.5	23.3	7.8	63.9	4.9	16	16
1	11	1	75	0	39.6	14.2	90.9	51.3	107.2	18.9	16.3	4.7	134.8	26.3	27.5	7.3	95.1	12.1	16	16
1	12	2	75	1	10.0	19.3	72.3	62.3	93.7	20.6	21.5	1.3	122.1	24.3	28.4	3.6	112.1	4.9	16	16
2	13	1	120	1	10.0	15.2	119.2	109.2	125.1	17.9	6.0	2.7	150.8	21.1	25.7	3.2	140.8	5.9	16	16
2	14	1	0	0	46.7	14.5	47.8	1.1	54.6	18.1	7.3	3.7	84.3	23.9	29.7	5.7	37.3	9.4	16	14
2	15	1	75	1	10.0	15.0	80.9	70.9	101.8	17.9	20.8	2.9	121.6	23.6	19.9	5.7	111.6	8.6	16	16
2	16	2	75	0	85.1	20.1	93.6	8.5	101.7	23.8	8.2	3.8	135.4	29.4	33.6	5.6	50.3	9.4	16	16
2	17	1	75	0	42.9	14.9	97.9	55.1	104.9	22.4	7.0	7.5	141.2	29.8	36.3	7.4	98.3	14.9	16	16
2	18	2	120	1	10.0	21.1	107.4	97.4	115.3	17.6	7.9	-3.5	148.8	23.9	33.6	6.2	138.8	2.8	16	16
2	19	1	0	1	10.0	13.4	39.7	29.7	43.9	10.0	3.9	-3.7	73.3	18.6	29.4	8.6	63.3	4.9	16	14
2	20	2	75	1	10.0	20.6	75.9	65.9	100.1	17.7	24.2	-2.8	124.8	25.0	24.7	7.3	114.8	4.5	16	15
2	21	2	120	0	77.1	19.1	115.0	37.9	125.3	19.2	10.3	0.2	159.7	25.8	34.3	6.5	82.6	6.7	16	16
2	22	2	0	1	10.0	20.5	31.0	21.0	32.1	8.0	-0.4	-12.7	51.8	16.1	19.7	8.1	41.8	-4.6	16	15
2	23	1	120	0	42.4	15.9	121.5	79.1	126.5	18.8	3.6	2.6	168.3	25.5	41.8	6.7	125.4	9.3	16	14
2	24	2	0	0	91.5	18.9	66.0	-25.5	67.1	14.0	1.1	-4.9	87.8	22.9	20.7	9.0	-3.7	4.1	16	16

Population: 1 German, 2 French. Treeshelter (cm), Stump: 0 not stumped, 1 stumped. Years: 1996 (96) to 1999 (99). H = height (cm), D = stem diameter (mm) at ground level, IH = increment of height (cm), ID = increment of stem diameter (mm), N = number of trees per plot.

APPENDIX IV Protocols for isozyme analyses

IV.1 Preparation of material for isozyme extraction.

Isozymes can be extracted from a wide range of plant parts, including leaves, imbibed seeds, fruits, roots, flowers, pollen and ovules. Four different sample sources from walnut have been used as extracts for isozyme analysis: young leaves (Aleta *et al.* 1990, Aleta *et al.* 1993, Arulsekar and Parfitt 1986, Arulsekar *et al.* 1985, Arulsekar *et al.* 1986, Germain *et al.* 1993, Solar *et al.* 1994), pollen (Solar *et al.* 1993), dormant buds (Malvolti *et al.* 1993) and seed embryos (Malvolti *et al.* 1995).

In laboratory pre-sessions, three sources of tissue extracts from locally sampled walnuts, were initially tested for isozyme analysis; seed embryos, bud meristems and young leaves. Extracts from seed embryos produced successful results with the resolution of fifteen enzyme systems: Aspartate aminotransferase (AAT) (E.C. 2.6.1.1.), Aconitase (ACO) (E.C. 4.2.1.3.), Acid phosphatase (ACP) (E.C. 3.1.3.2.), Alcohol dehydrogenase (ADH) (E.C. 1.1.1.1.), Aldolase (ALD) (E.C. 4.1.2.13.), β-Esterase (β-EST) (E.C. 3.1.1.-.), Glutamate dehydrogenase (GDH) (E.C. 1.4.1.2.), Glyceraldehyde-3-phosphate dehydrogenase (G3PDH) (E.C. 1.2.1.12.), Glucose-6-phosphate dehydrogenase (G6PDH) (E.C. 1.1.1.49.), Isocitrate (IDH) (E.C. 1.1.1.42.), Lactate dehydrogenase (LDH) (E.C. 1.1.1.27.), Phosphoglucose isomerase (PGI) (E.C. 5.3.1.9.), Phosphoglucose mutase (PGM) (E.C. 5.4.2.2.), Shikimate dehydrogenase (SKD) (E.C. 1.1.1.25.), and 6-Phosphogluconate dehydrogenase 6-PGD (E.C. 1.1.1.44.). Twenty loci were identified, of which four were polymorphic, although activity in five systems was unresolved. Analyses using bud meristems resulted in a similar number of identified loci but their resolutions were vastly inferior to those obtained from seed embryo extracts. Malate dehydrogenase (MDH) (E.C. 1.1.1.37.) was also tested but no activity was resolved with either tissue extract. An additional system, Peroxidase (PRX) (E.C. 1.11.1.7.), produced resolvable activity with extracts from young leaves.

However, the major drawback with both these systems was the destructive mode of sampling. In later analyses, embryo or bud meristem tissues were excluded, due to the absence of spare seed and the small size of the seedlings. For this research project, the need for a non-destructive sampling technique made young leaf material the most convenient source for isozyme extraction. Aleta *et al.* (1990) and Arulsekar and Parfitt (1986) recommended that young leaf material was the most convenient tissue for isozyme analysis in walnut. However, the leaves of many perennial fruit and nut trees contain varying amounts of phenolic compounds, leading to interference with enzyme activity detected on gels (Torres 1983).

Juglone, a naphthoquinone, is found in all *Juglans* species (Harborne and Turner 1984), therefore walnut leaf extracts can lead to poor enzyme resolution or 'ladies fingernails' in some systems. Aspartate aminotransferase, which was most effected by juglone levels, was tested with the addition of 3.5 mM dithiothreitol to the enzyme stain, in an attempt to resolve two loci just visible behind a dense mercaptoethanol front but resolution of this system failed to improve.

The young leaves selected for sampling were typically between 2 to 4 cm long and free from any insect, fungal and viral damage. Approximately 150 mm^2 of material was harvested from the trees at the field trial sites, and immediately processed *in situ*.

IV.2 Isozyme extraction

IV.2.1 Extraction buffer system

The extraction buffer is critical to the success of any isozyme extraction procedure. Many different extraction buffers have been used in different groups of organisms, and these have been summarised in a number of works (*e.g.* Cheliak and Pitel 1984, Wendel and Weeden 1990). Unfortunately as there is no one buffer that is effective in all species, a degree of experimentation was necessary to produce an effective extraction buffer for *Juglans regia*.

Extraction buffers were tested on three different tissue samples; seed embryos, bud meristems and young leaves, and with the 16 enzyme systems (Section IV.1). Three published extraction buffers (Aleta *et al.* 1990, Arulsekar *et al.* 1985, Arulsekar and Parfitt 1986) were tested and compared against an extraction buffer widely used across a range of species in the Department of Plant Sciences (Stephen Harris[1], pers. comm.). After initial indications that the in-house buffer produced the best resolution of enzyme activity across a range of systems, the buffer was further improved by the addition of 8% PVP-40T, included in the buffers of Aleta *et al.* (1990) and Weeden and Lamb (1985), and by excluding mercaptoethnol (as in the buffers of Arulsekar *et al.* (1985) and Arulsekar and Parfitt (1986)).

The extraction buffer used in assaying the walnut genotypes, after the experimentation phase was complete, was the following: 50 ml lithium borate gel buffer, 37 mg potassium chloride, 10 mg magnesium chloride, 18 mg EDTA tetrasodium, 25 mg PVPP, 0.5 ml Triton-X-100 and 4 g PVP-40T.

[1] Dr. Stephen Harris, Department of Plant Sciences, University of Oxford.

IV.2.2 Sample preparation

COLLECTION

1. A small amount (approximately 150 mm^2) of young leaf material was harvested, placed directly into 1.5 ml Eppendorf tubes and placed in a rack which was kept on ice.
2. 5 µl cold extraction buffer (kept on ice) was added to each Eppendorf tube and the material was ground into a homogeneous paste using a brass rod. The rod was rinsed in water and dried between sample extractions.
3. Once isozyme extracts had been made, they were immediately frozen in liquid nitrogen for transportation back to the laboratory where they were stored at -80 ^oC.

PREPARATION FOR ELECTROPHORESIS

4. Extracts were thawed slowly by placing the Eppendorf tubes on ice at room temperature for 45 minutes.
5. Samples were centrifuged at 13,000 rpm for one minute at room temperature to pellet debris.
6. A 3 × 8 mm wick, cut from whatman 3 MM filter paper, was placed into each Eppendorf tube and the sample was absorbed into the filter paper. The prepared tubes were kept on ice for a short time while the gel was prepared for loading samples.

IV.2.3 Starch gel electrophoresis.

Many gel and buffer systems exist (Scandalios 1969, Shields *et al.* 1983). Three gel and buffer systems were assayed in laboratory pre-sessions: histidine citrate, lithium borate and tris citrate (Soltis and Soltis 1990) (Table IV.1). Histidine citrate was found to be most effective for all enzyme systems with young leaf material, except for AAT where lithium borate buffer gave better resolution.

The choice of starch as a medium was due to its ease of use, the large number of samples that could be analysed at one time and the wide range of gel strengths that could be prepared. A 13 % starch gel was found to the most effective for this study, although starch gels can be made between 9 % and 14 % (Wendel and Weeden 1990).

A technique for preparing the starch gels, using a microwave oven, was developed for this study which proved to be considerably easier, and more consistent in quality, to the traditional method using direct heat from a Bunsen burner. It was also found that when the method

Table IV.1 Gel and electrode buffer systems tested with walnut material, modified from Soltis and Soltis (1990).

Histidine citrate pH 7.5	**Lithium borate pH 8.6**	**Tris citrate pH 7.5**
Gel buffer 8.36 g histidine HCl 0.03 g EDTA (disodium) 1 litre deionised H_20	**Gel buffer** 5.40 g tris base 1.28 g citric Acid 100ml electrode buffer 900ml deionised H_20	**Gel buffer** 67.5 ml electrode buffer 932.5 ml deionised H_20
Electrode buffer 15.1 g tris base 7.3 g citric acid 1 litre deionised H_20	**Electrode buffer** 1.2 g lithium hydroxide 12.5 g boric acid 1 litre deionised H_20	**Electrode buffer** 16.35 g tris-base 6.2 g citric acid 1 litre deionised water

described below was closely adhered to, the evacuation of air bubbles from the starch mixture, normally required by using a vacuum pump, was unnecessary.

1. 13% starch gels were prepared by adding 52 g electrophoresis grade hydrolysed potato starch (S4501) to 100 ml gel buffer in a 250 ml flask and the mixture suspended by gentle shaking. Preparation of this suspension up to 12 hours prior to the next stage was found to be beneficial in promoting the uniform suspension on the starch, thereby reducing poorly mixed gels.
2. 300 ml gel buffer was separately added to a 1 L Buchner flask and heated in a microwave oven on full power (800 watts) for 3 minutes, or until boiling.
3. The starch mixture in the 250 ml flask was thoroughly shaken to ensure a uniform suspension immediately prior to being added, in one movement, to the boiling 300 ml gel buffer in the 1 L Buchner flask. The contents of this flask were kept moving while the starch mixture was added and swirled vigorously once complete, to ensure thorough mixing. The now combined mixture was returned to the microwave oven and heated for a further 40 seconds until the suspension thickened: transforming from a white to greyish colour and thinning very slightly. It was important to ensure that the starch mixture was not over-cooked.
4. After gentle swirling of the mixture to allow large air bubbles to escape, the starch mixture was quickly poured into the centre of a glass gel mould (240 × 125 × 8 mm), using a single action, ensuring that no air bubbles entered the matrix. Air bubbles or any lumps in the gel could be removed with a Pasteur pipette. A glass plate was placed on top of the mould and gentle pressure was applied until the mould was completely filled with the starch mixture. The gel was allowed to set at room temperature for about 10 minutes and then placed at 4 ^{o}C to cool completely.

5. Once the gel was completely cool the top plate was removed with a blunt sectioning razor. A scalpel blade was used to separate the edge of the gel from the side of the mould and any excess starch was wiped from the edge of the mould. A cut was made in the gel, between a pair of markers placed 4 cm from the bottom of the gel mould, to form a well for the sample wicks.
6. The wicks were inserted vertically into the gel approximately 1.5 mm apart from each other, which allowed approximately 60 samples to be loaded per gel. A drop of 1% aqueous bromophenol blue was placed onto the surface of the first and last wicks once they were loaded. The surface of the gel was covered with plastic cling film.
7. The gel mould was then placed into the electrophoresis apparatus and each half of the apparatus filled with electrode buffer. The gel and buffer were connected using cellulose sponges, such that the tracker dye moves towards the positive electrode. Ice packs (which were removed from the freezer approximately one half-hour prior to use) were placed on top of the gel and the apparatus was put into a refrigerator at 4 ^{o}C. After one hour the wicks were removed from the gel and a glass rod inserted between the gel and the mould at the cathodal edge. This was found to improve the electrical contact between the cathodal and anodal slices.
8. With the histidine citrate system, gels were run at 130 mA current and 320 volts for 6 to 7 hours. With lithium borate (for AAT), gels were run at 70 mA current and 250 volts for $3^{1}/_{2}$ to 4 hours. These timings allowed the bromophenol blue marker to migrate approximately 8 cm from the origin.

IV.3 Gel slicing

Staining of enzyme activity in starch gels was undertaken on 1 mm horizontal slices cut from the main gel.

1. The gel mould was removed from the electrophoresis apparatus and the gel cut at the position of the bromophenol blue marker to allow the removal of the central (anodal) portion of the gel.

2. The gel was placed on a dry, 3 mm glass plate and seven 1 mm plastic strips were placed on each side of the gel, to act as guides for slicing, and the gel covered with a piece of 6 mm glass. A length of nylon fishing line, pulled taught between the hands, was pulled through the gel using the plastic strips as guides. The top slice of gel was discarded and one plastic strip removed from each side to allow a repeat of the slicing process. The above process was repeated producing six slices, leaving the bottom 1 mm slice of the gel which was discarded. Each 1 mm starch slice was placed into a shallow glass tray and kept at 4 ^{o}C

until required for staining.

IV.4 Enzyme staining

Many different staining recipes are available in the literature, as reviewed by Soltis and Soltis (1990). Sixteen enzyme systems were tested with all of the different sample origins of walnut and with the three buffer systems detailed above: AAT, ACO, ACP, ADH, ALD, β-EST, GDH, G-3-PDH, G-6-PDH, IDH, LDH, MDH, PGI, PGM, SKD, and 6-PGD. Fifteen of these showed some activity with sample extracts from seed embryo and bud meristem material but MDH activity was not revealed with any of the sample extracts or buffer systems. With extracts from young leaf material, only five of the above systems had consistent resolvable activity (AAT, ACO, IDH, PGI and PGM) but an additional system, PRX was tested and activity revealed.

The stains were incubated at approximately 37 ^{o}C in the dark, except AAT and PRX, which were incubated at room temperature in the dark. After the required incubation time, which varied between systems, the staining solution was discarded and replaced with 50% glycerol. The gels were left in the glycerol at 4 ^{o}C for approximately 6-8 hours before scoring and photographing. This process resulted in clearer resolved gels with some systems (AAT, PGI and PGM).

Staining times depended on factors such as the concentration and activity of the enzyme in the extract. Some systems, *e.g.* AAT and PGI, stained very quickly, whilst others, *e.g.* PGM, stained very slowly.

ENZYME STAINS (modified from Soltis and Soltis 1990)

Acid phosphatase ACP E. C. 3.1.3.2.

Buffer:	50 ml 0.4 M sodium acetate buffer, pH 5.0 (pre-soak).
	50 ml 0.2 M sodium acetate buffer, pH 5.0.
Substrate:	50 mg α-napthyl acid phosphate (N7000).
Dye:	50 mg KK fast black K salt (F7253).
	0.5 ml 10% $MgCl_2$ (M8266).
Structure:	monomer, dimer.

Aconitase ACO E. C. 4.2.1.3.

Buffer: 50 ml 0.2 M Tris-HCl, pH 8.0.
Substrate: 50 mg cis-aconitic acid (A3412).
30 units IDH (I2516).
Dye: 10 mg NADP (N0505).
10 mg MTT (M2128).
2 mg PMS (P9625).
0.5 ml 10% $MgCl_2$ (M8266).
Structure: monomer.

Aldolase ALD E. C. 4.1.2.13.

Buffer: 50 ml 0.05 M Tris-HCl, pH 8.0
Substrate: 200 mg fructose-1,6-bisphosphate, sodium salt (F4757).
75 mg sodium arsenate (S9663).
Dye: 10 mg NAD (N7004).
10 mg MTT (M2128).
2 mg PMS (P9625).
Structure: tetramer.

Alcohol dehydrogenase ADH E. C. 1.1.1.1.

Buffer: 50 ml 0.05 M Tris-HCl, pH 8.0.
Substrate: 0.2 ml ethanol (BDH).
Dye: 10 mg NAD (N7004).
10 mg MTT (M2128).
2 mg PMS (P9625).
Structure: dimer.

Aspartate aminotransferase AAT E. C. 2.6.1.1.

Buffer: 50 ml 0.1 M Tris-HCl, pH 8.5.
Substrate: 18 mg α-ketoglutaric acid (K1750).
65 mg L-aspartic acid (A9256).
Dye: 5 mg pyridoxal-5-phosphate (P9255).
250 mg PVP-40T.
50 mg disodium EDTA (ED2SS).
710 mg Na_2HPO_4 (BDH).
200 mg fast blue BB salt (F3378).
Structure: dimer.

ß-Esterase ß-EST E.C. 3.1.1.-.

Buffer: 20 ml H_2O.
20 ml 0.2 M NaH_2PO_4 (BDH).
10 ml 0.2 M Na_2HPO_4 (BDH).
Substrate: 2 ml 1% sodium ß-napthyl acetate (N6875) in acetone (BDH).
Dye: 125 mg fast blue RR salt (F0500).
1 ml acetone (BDH).
Structure: monomer, dimer.

Glucose-6-phosphate dehydrogenase G-6-PDH E. C. 1.1.1.49.

Buffer: 50 ml 0.05 M Tris-HCl, pH 8.0.
Substrate: 50 mg glucose-6-phosphate, disodium salt (G7250).
Dye: 5 mg NADP (N0505).
10 mg MTT (M2128).
2 mg PMS (P9625).
0.5 ml 10% $MgCl_2$ (M8266).

Structure: dimer.

Glyceraldehyde-3-phosphate dehydrogenase G-3-PDH E. C. 1.2.1.12.
Buffer: 50 ml 0.1 M Tris-HCl, pH 7.5.
Substrate: 45 mg monosodium fructose-1,6-bisphosphate (F4757).
10 units aldolase (A8811)
Dye: 10 mg NADP (N0505).
15 mg MTT (M2128).
1 mg PMS (P9625).
0.5 ml 10% $MgCl_2$ (M8266).
Structure: tetramer.

Glutamate dehydrogenase GDH E. C. 1.4.1.2.
Buffer: 50 ml 0.1 M Tris-HCl, pH 8.5.
Substrate: 210 mg L-monosodium glutamate (G1626).
Dye: 10 mg NAD (N7004).
10 mg MTT (M2128).
1 mg PMS (P9625).
25 mg ATP (A2383).
Structure: hexamer.

Isocitrate dehydrogenase IDH E. C. 1.1.1.42.
Buffer: 50 ml 0.1 M Tris-HCl, pH 8.0.
Substrate: 75 mg trisodium isocitrate (I1252).
Dye: 10 mg NADP (N0505).
10 mg MTT (M2128).
3 mg PMS (P9625).
0.5 ml 10% $MgCl_2$ (M8266).
Structure: dimer.

Lactate dehydrogenase LDH E. C. 1.1.1.27.
Buffer: 50 ml 0.05 M Tris-HCl, pH 8.0.
Substrate: 100 mg lactic acid, lithium salt (L2250).
Dye: 10 mg NAD (N7004).
10 mg MTT (M2128).
2 mg PMS (P9625).
Structure: tetramer.

Malate dehydrogenase MDH E. C. 1.1.1.37.
Buffer: 50 ml 0.1 M Tris-HCl, pH 7.5.
Substrate: 850 mg L-malic acid (M0875).
Dye: 12 mg NAD (N7004).
10 mg MTT (M2128).
3 mg PMS (P9625).
Structure: dimer.

Peroxidase PRX E.C. 1.11.1.7.
Buffer: 50 ml 0.2 M acetate buffer, pH 5.0.
1 ml $CaCl_2$.
Substrate: 0.5 ml 30% hydrogen peroxide (H1009).
Dye: 40 mg 3-amino-9-ethyl-carbazol (A5754).
5 ml N, N-dimethyl formamide (D4254).
Structure: monomer.

6-Phosphogluconate dehydrogenase 6-PGD E. C. 1.1.1.44.

Buffer: 50 ml 0.1 M Tris-HCl, pH 8.0.
Substrate: 50 mg trisodium 6-phospho-gluconate (P7877).
Dye: 10 mg NADP (N0505).
15 mg MTT (M2128).
3 mg PMS (P9625).
0.5 ml 10% $MgCl_2$ (M8266).
Structure: monomer, dimer.

Phosphoglucose isomerase PGI E. C. 5.3.1.9.

Buffer: 50 ml 0.1 M Tris-HCl, pH 7.5.
Substrate: 20 mg disodium fructose-6-phosphate (F3627).
10 units glucose-6-phosphate dehydrogenase (G4134).
Dye: 7 mg NADP (N0505).
12 mg MTT (M2128).
3 mg PMS (P9625).
0.5 ml 10% $MgCl_2$ (M8266).
Structure: dimer.

Phosphoglucose mutase PGM E. C. 2.7.5.1.

Buffer: 50 ml 0.1 M Tris-HCl, pH 7.5.
Substrate: 80 mg disodium glucose-1-phosphate (G7000).
20 units glucose-6-phosphate dehydrogenase (G4134).
Dye: 10 mg NADP (N0505).
15 mg MTT (M2128).
1 mg PMS (P9625).
15 mg ATP (A2383).
0.5 ml 10% $MgCl_2$ (M8266).
Structure: monomer.

Shikimate dehydrogenase SKD E. C. 1.1.1.25.

Buffer: 50 ml 0.1 M Tris-HCl, pH8.5
Substrate: 50 mg Shikimic acid (S5375)
Dye: 5 mg NADP (N0505).
10 mg MTT (M2128).
2 mg PMS (P9625).
Structure: monomer.

The solutions of 10 mg/ml MTT, 10 mg/ml PMS, 10 mg/ml NADP, 10 mg/ml NAD and 10% $MgCl_2$ were conveniently premixed in batches and stored at 4 ^{o}C for up to 6 months. Isocitrate dehydrogenase (IDH) solutions, for ACO staining, were conveniently made up in aliquots of 40 units/ml and stored at –20 ^{o}C until needed. G-6-PDH solutions at 1 unit/µl were stored at 4 ^{o}C.

Site	Block	Prov	meanH98	meanD98	varH98	varD98	meanH99	meanD99	meanIH99	meanID99	varH99	varD99	varIH99	varID99	N98	N99	Surv98-99
1	1	1	11.000	10.000	*	*	63.000	17.000	52.000	7.000	*	*	*	*	1	1	100.000
1	1	2	7.800	8.000	26.325	23.000	24.700	11.600	16.900	3.600	283.950	33.300	139.675	8.300	5	5	100.000
1	1	3	11.071	11.857	7.786	34.810	33.857	15.429	22.786	3.571	377.726	29.952	356.321	8.286	7	7	100.000
1	1	4	10.750	10.375	18.286	11.411	47.125	17.375	36.375	7.000	209.768	14.554	214.839	3.429	8	8	100.000
1	1	5	9.714	8.143	3.988	8.810	23.571	11.857	13.857	3.714	145.119	12.476	159.060	14.238	7	7	100.000
1	1	6	14.200	13.900	21.733	19.878	44.200	17.300	30.000	3.400	572.678	13.567	576.333	5.600	10	10	100.000
1	1	7	9.500	8.571	11.833	10.286	38.714	15.429	29.214	6.857	275.821	24.952	239.321	13.810	7	7	100.000
1	1	8	9.125	8.250	8.411	11.929	26.643	12.429	16.571	3.286	75.226	15.619	78.369	19.238	8	7	87.500
1	1	9	13.250	8.700	52.181	6.233	42.650	14.300	29.400	5.600	317.558	9.789	405.433	4.489	10	10	100.000
1	1	10	11.091	9.545	14.341	7.673	32.455	13.636	21.364	4.091	229.023	16.455	177.005	5.291	11	11	100.000
1	1	11	9.545	9.091	18.373	34.091	30.045	13.091	20.500	4.000	379.173	26.291	263.900	6.800	11	11	100.000
1	1	12	29.250	16.500	3.125	4.500	88.750	22.000	59.500	5.500	6.125	50.000	0.500	24.500	2	2	100.000
1	1	13	15.250	15.000	0.125	8.000	90.500	23.500	75.250	8.500	72.000	12.500	66.125	0.500	2	2	100.000
1	1	14	22.000	12.000	*	*	89.000	18.000	67.000	6.000	*	*	*	*	1	1	100.000
1	1	15	16.500	19.500	0.500	4.500	74.750	28.500	58.250	9.000	351.125	12.500	378.125	32.000	2	2	100.000
1	1	16	13.500	8.250	10.833	4.250	70.625	17.000	57.125	8.750	701.229	18.667	567.896	30.917	4	4	100.000
1	1	17	18.750	17.000	1.125	2.000	58.250	19.000	39.500	2.000	1176.125	18.000	1104.500	8.000	2	2	100.000
1	1	18	8.000	7.000	2.000	0.000	36.000	11.000	28.000	4.000	450.000	2.000	512.000	2.000	2	2	100.000
1	2	1	10.000	19.000	*	*	82.000	20.000	72.000	1.000	*	*	*	*	1	1	100.000
1	2	2	12.800	11.800	13.825	8.200	31.000	13.600	18.200	1.800	179.375	5.800	160.325	6.700	5	5	100.000
1	2	3	12.125	12.375	4.554	6.839	46.938	15.375	34.813	3.000	470.388	5.982	443.138	9.143	8	8	100.000
1	2	4	14.813	13.875	144.924	62.411	42.063	14.250	27.250	0.375	772.388	12.786	408.714	34.554	8	8	100.000
1	2	5	11.429	11.571	16.619	9.619	45.643	15.143	34.214	3.571	263.393	7.143	227.071	6.286	7	7	100.000
1	2	6	11.750	14.300	40.847	38.456	35.850	13.800	24.100	-0.500	398.669	20.400	273.600	7.167	10	10	100.000
1	2	7	9.357	8.857	15.310	14.476	28.143	13.143	18.786	4.286	236.143	11.143	225.238	0.905	7	7	100.000
1	2	8	9.750	11.500	4.571	25.143	38.188	13.125	28.438	1.625	246.281	9.554	216.460	11.411	8	8	100.000
1	2	9	11.950	12.300	15.858	38.456	53.750	15.200	41.800	2.900	794.181	46.400	606.178	10.544	10	10	100.000
1	2	10	10.182	11.000	5.864	15.800	51.318	14.818	41.136	3.818	400.314	14.164	370.455	7.964	11	11	100.000
1	2	11	9.773	11.091	7.118	14.691	50.045	13.818	40.273	2.727	375.573	19.364	307.518	13.418	11	11	100.000
1	2	12	14.250	19.000	66.125	32.000	85.250	23.000	71.000	4.000	6.125	2.000	32.000	18.000	2	2	100.000
1	2	13	9.000	13.500	2.000	40.500	58.000	15.500	49.000	2.000	2.000	0.500	0.000	32.000	2	2	100.000
1	2	14	21.500	16.000	*	*	82.000	19.000	60.500	3.000	*	*	*	*	1	1	100.000
1	2	15	6.500	7.000	4.500	2.000	48.750	11.500	42.250	4.500	1128.125	4.500	1275.125	0.500	2	2	100.000
1	2	16	19.167	19.333	46.083	22.333	97.000	20.333	77.833	1.000	147.000	12.333	116.083	39.000	3	3	100.000
1	2	17	8.250	10.000	28.125	72.000	24.500	12.500	16.250	2.500	612.500	112.500	378.125	4.500	2	2	100.000
1	2	18	13.750	11.500	91.125	12.500	54.750	15.000	41.000	3.500	903.125	32.000	420.500	4.500	2	2	100.000
1	3	1	11.000	13.000	18.000	128.000	45.000	14.500	34.000	1.500	450.000	24.500	288.000	40.500	2	2	100.000
1	3	2	8.500	7.400	5.000	5.300	32.800	12.000	24.300	4.600	410.325	14.500	349.450	16.300	5	5	100.000
1	3	3	10.063	11.250	8.031	37.643	39.000	12.875	28.938	1.625	202.500	6.982	189.603	17.982	8	8	100.000
1	3	4	8.875	9.750	3.625	9.071	37.563	11.875	28.688	2.125	202.888	8.696	183.210	1.268	8	8	100.000
1	3	5	10.429	8.429	25.036	7.952	38.286	12.286	27.857	3.857	129.321	2.238	142.310	8.143	7	7	100.000
1	3	6	11.750	9.800	29.903	16.400	35.900	13.300	24.150	3.500	282.767	12.678	169.058	9.389	10	10	100.000
1	3	7	9.929	9.571	1.952	9.952	41.571	11.286	31.643	1.714	198.536	4.238	197.393	2.571	7	7	100.000
1	3	8	8.250	7.000	9.857	14.571	28.313	10.125	20.063	3.125	136.710	4.125	145.960	4.696	8	8	100.000
1	3	9	10.450	9.400	12.025	16.044	53.300	13.800	42.850	4.400	327.622	15.956	256.614	3.600	10	10	100.000
1	3	10	11.917	10.500	9.947	29.364	45.333	12.333	33.417	1.833	395.697	13.152	359.311	8.879	12	12	100.000
1	3	11	10.450	9.500	5.858	22.722	47.000	13.400	36.550	3.900	153.000	6.267	155.692	9.656	10	10	100.000
1	3	12	25.750	18.000	351.125	32.000	90.750	22.000	65.000	4.000	231.125	2.000	1152.000	18.000	2	2	100.000
1	3	13	12.000	15.000	50.000	200.000	76.000	17.500	64.000	2.500	0.000	12.500	50.000	112.500	2	2	100.000
1	3	14	15.000	10.000	*	*	53.500	16.000	38.500	6.000	*	*	*	*	1	1	100.000
1	3	15	13.750	11.000	28.125	72.000	70.500	16.500	56.750	5.500	4.500	24.500	55.125	12.500	2	2	100.000
1	3	16	14.500	10.667	117.250	42.333	56.000	13.333	41.500	2.667	1227.000	49.333	618.250	0.333	3	3	100.000
1	3	17	11.250	7.000	36.125	8.000	54.250	17.500	43.000	10.500	1035.125	84.500	684.500	40.500	2	2	100.000
1	3	18	5.000	3.000	*	*	12.500	6.000	7.500	3.000	*	*	*	*	1	1	100.000
1	4	1	3.500	4.000	*	*	30.500	9.000	27.000	5.000	*	*	*	*	1	1	100.000
1	4	2	11.400	10.000	10.175	5.500	40.900	13.600	29.500	3.600	276.925	3.300	210.750	3.300	5	5	100.000
1	4	3	10.438	9.500	10.174	10.000	31.625	13.125	21.188	3.625	302.411	16.125	310.424	6.839	8	8	100.000
1	4	4	10.000	11.375	10.643	16.554	43.938	14.500	33.938	3.125	259.888	9.714	267.317	4.125	8	8	100.000
1	4	5	14.214	9.143	40.488	5.810	40.786	12.429	26.571	3.286	253.655	7.619	240.119	2.905	7	7	100.000
1	4	6	12.500	10.700	6.889	7.567	41.889	15.111	29.111	4.000	204.111	4.861	158.924	2.750	10	9	90.000
1	4	7	10.786	8.571	9.405	9.619	41.429	12.857	30.643	4.286	378.702	10.143	322.393	3.238	7	7	100.000
1	4	8	9.750	7.750	5.071	1.643	29.000	11.250	19.250	3.500	224.286	5.071	208.143	2.000	8	8	100.000
1	4	9	13.100	10.300	38.656	12.233	44.444	15.222	30.500	4.333	354.153	0.944	361.750	8.250	10	9	90.000
1	4	10	10.227	7.636	10.918	5.855	37.182	11.727	26.955	4.091	183.814	11.818	151.623	5.691	11	11	100.000
1	4	11	10.250	7.600	15.958	7.378	40.050	11.900	29.800	4.300	226.303	4.544	139.289	3.789	10	10	100.000
1	4	12	11.000	13.000	8.000	0.000	82.250	19.000	71.250	6.000	1.125	0.000	15.125	0.000	2	2	100.000
1	4	13	14.250	15.000	28.125	98.000	58.250	22.000	44.000	7.000	1653.125	72.000	1250.000	2.000	2	2	100.000
1	4	14	18.000	16.000	*	*	81.000	23.000	63.000	7.000	*	*	*	*	1	1	100.000
1	4	15	8.667	7.333	8.583	0.333	42.500	12.000	33.833	4.667	424.750	9.000	319.083	9.333	3	3	100.000
1	4	16	11.200	12.000	21.700	43.000	60.100	16.200	48.900	4.200	533.550	45.700	355.550	0.700	5	5	100.000
1	4	17	7.000	9.000	*	*	24.000	12.000	17.000	3.000	*	*	*	*	1	1	100.000
1	4	18	10.000	15.000	*	*	54.500	15.000	44.500	0.000	*	*	*	*	1	1	100.000
1	5	1	6.000	7.000	*	*	32.000	9.000	26.000	2.000	*	*	*	*	1	1	100.000
1	5	2	7.700	7.000	9.575	11.500	27.250	11.250	18.375	3.250	171.583	14.250	127.063	11.583	5	4	80.000
1	5	3	11.125	11.250	6.125	24.500	39.625	13.250	28.500	2.000	338.482	23.357	301.429	3.143	8	8	100.000
1	5	4	10.188	14.000	5.353	43.714	41.250	15.625	31.063	1.625	451.929	39.125	393.031	3.411	8	8	100.000
1	5	5	9.429	11.857	12.702	52.143	29.571	10.286	20.143	-1.571	248.036	8.905	268.726	31.952	7	7	100.000
1	5	6	11.200	10.600	18.956	12.044	41.111	13.556	29.333	2.444	332.611	5.778	437.375	6.778	10	9	90.000
1	5	7	13.143	14.286	32.226	29.905	43.917	15.167	30.417	-0.167	127.542	2.967	189.542	26.167	7	6	85.714
1	5	8	8.563	8.500	3.031	6.000	40.063	11.750	31.500	3.250	181.674	4.214	187.214	3.929	8	8	100.000
1	5	9	9.950	10.000	9.303	35.333	34.950	12.200	25.000	2.200	355.025	17.067	288.389	9.733	10	10	100.000
1	5	10	9.045	10.273	9.573	39.418	40.778	13.889	30.944	2.222	368.569	19.111	299.840	18.694	11	9	81.818
1	5	11	10.773	9.273	12.118	12.218	36.909	11.455	26.136	2.182	195.241	8.073	140.005	2.164	11	11	100.000
1	5	12	16.000	23.500	8.000	180.500	83.750	24.000	67.750	0.500	190.125	18.000	120.125	84.500	2	2	100.000
1	5	13	14.250	17.000	6.125	18.000	91.250	19.500	77.000	2.500	351.125	4.500	264.500	4.500	2	2	100.000
1	5	14	12.500	12.000	*	*	42.500	15.000	30.000	3.000	*	*	*	*	1	1	100.000
1	5	15	11.250	12.000	15.125	50.000	63.750	17.500	52.500	5.500	15.125	24.500	0.000	4.500	2	2	100.000
1	5	16	14.250	11.250	62.083	50.917	48.750	14.500	34.500	3.250	766.417	36.333	504.167	2.250	4	4	100.000
1	5	17	12.250	13.000	0.125	18.000	54.750	15.500	42.500	2.500	45.125	4.500	50.000	4.500	2	2	100.000
1	5	18	8.000	10.000	*	*	28.000	13.000	20.000	3.000	*	*	*	*	1	1	100.000
1	6	1	12.500	14.000	*	*	53.000	16.000	40.500	2.000	*	*	*	*	1	1	100.000
1	6	2	10.600	9.400	14.300	36.300	38.375	10.000	27.250	0.250	443.229	8.667	439.417	20.250	5	4	80.000
1	6	3	11.214	12.000	9.321	20.667	53.786	14.429	42.571	2.429	709.488	12.286	587.286	15.619	7	7	100.000
1	6	4	11.125	11.125	19.411	16.696	48.563	12.375	37.438	1.250	258.888	11.125	210.817	6.786	8	8	100.000
1	6	5	9.571	9.571	7.702	17.952	37.500	11.571	27.929	2.000	177.750	13.286	147.202	1.333	7	7	100.000

Sites: 1= Oxon, 2 = Gloucestershire , 3 = Somerset. Prov = provenance, 98 and 99 = years, H = height (cm), D = stem diameter (mm), IH = height increment (cm), ID = stem diameter increment (mm), var = variance, N = number of trees per plot, Surv = survival %.

Site	Block	Prov	meanH98	meanD98	varH98	varD98	meanH99	meanD99	meanIH99	meanID99	varH99	varD99	varIH99	varID99	N98	N99	Surv98-99
1	6	6	14.100	11.300	13.322	21.789	38.450	12.900	24.350	1.600	259.081	9.433	240.725	13.600	10	10	100.000
1	6	7	9.357	9.000	4.393	8.333	29.286	12.286	19.929	3.286	281.488	6.238	255.202	1.571	7	7	100.000
1	6	8	9.375	12.125	16.411	17.268	43.071	14.571	33.643	1.714	426.619	4.952	367.393	18.238	8	7	87.500
1	6	9	12.300	15.000	3.567	19.778	53.389	16.667	41.278	2.111	232.549	11.500	237.194	8.361	10	9	90.000
1	6	10	11.227	13.091	9.268	43.691	46.818	15.273	35.591	2.182	473.164	27.218	386.691	24.764	11	11	100.000
1	6	11	11.136	10.455	11.905	16.873	40.318	12.636	29.182	2.182	217.964	8.455	188.014	4.164	11	11	100.000
1	6	12	19.750	16.000	0.125	8.000	77.750	17.500	58.000	1.500	105.125	0.500	112.500	4.500	2	2	100.000
1	6	13	9.500	10.500	0.500	12.500	59.000	13.000	49.500	2.500	288.000	0.000	264.500	12.500	2	2	100.000
1	6	14	14.500	17.000	*	*	90.000	18.000	75.500	1.000	*	*	*	*	1	1	100.000
1	6	15	13.000	10.000	72.000	50.000	50.000	13.000	37.000	3.000	2312.000	32.000	1568.000	2.000	2	2	100.000
1	6	16	8.000	10.250	15.333	16.250	50.750	13.000	42.750	2.750	947.417	2.000	1164.750	8.250	4	4	100.000
1	6	17	13.750	19.000	0.125	50.000	38.500	16.000	24.750	-3.000	364.500	32.000	351.125	2.000	2	2	100.000
1	6	18	20.750	17.500	0.125	4.500	76.500	20.500	55.750	3.000	2.000	0.500	1.125	2.000	2	2	100.000
1	7	1	6.000	5.000	*	*	25.000	9.000	19.000	4.000	*	*	*	*	1	1	100.000
1	7	2	8.500	8.200	6.500	7.700	38.300	12.600	29.800	4.400	482.200	10.300	401.575	2.300	5	5	100.000
1	7	3	9.938	8.500	7.388	5.429	46.813	13.875	36.875	5.375	358.853	7.554	297.482	10.839	8	8	100.000
1	7	4	12.063	12.125	27.674	40.125	48.563	15.125	36.500	3.000	1030.603	26.125	846.357	5.714	8	8	100.000
1	7	5	10.857	11.429	2.893	11.952	49.286	15.714	38.429	4.286	285.821	18.238	251.952	7.238	7	7	100.000
1	7	6	10.450	10.250	6.692	6.681	43.900	14.500	33.450	4.250	372.489	11.389	359.192	17.569	10	10	100.000
1	7	7	10.357	10.857	2.476	30.143	46.000	13.429	35.643	2.571	183.500	13.286	150.810	40.619	7	7	100.000
1	7	8	10.063	8.875	6.317	23.268	39.000	12.375	28.938	3.500	583.500	18.554	500.674	2.000	8	8	100.000
1	7	9	10.591	9.364	5.041	10.455	42.364	12.545	31.773	3.182	340.155	6.873	283.968	2.564	11	11	100.000
1	7	10	9.650	8.100	16.614	16.544	25.450	11.900	15.800	3.800	103.525	17.656	50.289	1.956	10	10	100.000
1	7	11	9.750	8.200	12.569	7.956	33.700	12.400	23.950	4.200	313.122	7.156	237.358	1.956	10	10	100.000
1	7	12	9.500	10.500	24.500	4.500	55.750	13.500	46.250	3.000	1128.125	0.500	820.125	8.000	2	2	100.000
1	7	13	15.500	14.500	32.000	4.500	54.250	15.000	38.750	0.500	1225.125	18.000	1653.125	40.500	2	2	100.000
1	7	14	13.000	17.000	*	*	82.500	18.000	69.500	1.000	*	*	*	*	1	1	100.000
1	7	15	16.000	12.500	50.000	12.500	72.250	19.000	56.250	6.500	231.125	18.000	66.125	0.500	2	2	100.000
1	7	16	16.250	10.250	148.750	35.583	46.250	14.000	30.000	3.750	273.417	44.667	83.833	2.917	4	4	100.000
1	7	17	44.000	12.000	2048.000	98.000	74.000	18.500	30.000	6.500	2450.000	84.500	18.000	0.500	2	2	100.000
1	7	18	23.500	25.000	*	*	73.000	29.000	49.500	4.000	*	*	*	*	1	1	100.000
1	7	19	6.000	5.000	*	*	*	*	*	*	*	*	*	*	1	0	0.000
1	8	1	13.000	8.000	*	*	56.500	12.000	43.500	4.000	*	*	*	*	1	1	100.000
1	8	2	10.100	9.600	16.675	36.800	31.750	14.250	20.000	2.750	8.250	4.250	12.167	44.250	5	4	80.000
1	8	3	10.375	8.750	20.411	15.357	45.313	12.750	34.938	4.000	234.638	16.500	216.531	13.714	8	8	100.000
1	8	4	10.000	11.375	1.071	10.554	47.750	14.750	37.750	3.375	247.143	3.071	231.857	13.411	8	8	100.000
1	8	5	12.857	12.429	11.226	18.619	54.357	15.429	41.500	3.000	519.976	20.952	505.333	31.667	7	7	100.000
1	8	6	13.100	12.000	26.878	14.667	43.950	15.300	30.850	3.300	296.914	8.678	228.892	9.344	10	10	100.000
1	8	7	11.143	14.429	14.726	20.952	47.429	14.571	36.286	0.143	325.952	19.952	213.821	15.143	7	7	100.000
1	8	8	8.688	9.000	4.424	18.286	31.813	11.875	23.125	2.875	110.781	7.554	85.982	19.554	8	8	100.000
1	8	9	11.227	10.000	5.518	11.200	52.545	14.727	41.318	4.727	259.073	12.618	237.964	17.218	11	11	100.000
1	8	10	10.042	9.000	8.703	11.636	40.042	12.417	30.000	3.417	83.975	3.174	91.727	9.720	12	12	100.000
1	8	11	11.300	10.900	11.456	14.767	47.800	14.100	36.500	3.200	162.844	15.433	102.222	14.178	10	10	100.000
1	8	12	24.000	15.000	50.000	32.000	86.000	22.500	62.000	7.500	144.500	0.500	24.500	24.500	2	2	100.000
1	8	13	11.750	12.500	3.125	4.500	89.250	15.500	77.500	3.000	136.125	0.500	98.000	2.000	2	2	100.000
1	8	14	10.500	12.000	*	*	67.000	15.000	56.500	3.000	*	*	*	*	1	1	100.000
1	8	15	13.000	17.000	72.000	0.000	63.500	18.000	50.500	1.000	180.500	72.000	480.500	72.000	2	2	100.000
1	8	16	7.000	8.667	13.000	64.333	42.000	14.667	35.000	6.000	1231.750	65.333	1004.250	3.000	3	3	100.000
1	8	17	15.000	11.500	0.500	12.500	70.750	16.500	55.750	5.000	78.125	4.500	66.125	2.000	2	2	100.000
1	8	18	8.000	9.000	*	*	16.000	14.000	8.000	5.000	*	*	*	*	1	1	100.000
1	9	1	2.500	8.000	*	*	41.000	15.000	38.500	7.000	*	*	*	*	1	1	100.000
1	9	2	11.200	9.000	45.825	31.500	39.200	13.000	28.000	4.000	447.575	17.500	300.000	5.000	5	5	100.000
1	9	3	11.438	9.250	4.388	7.643	46.188	13.750	34.750	4.500	147.496	6.786	124.786	4.571	8	8	100.000
1	9	4	8.813	8.750	13.067	18.500	42.875	12.375	34.063	3.625	296.911	15.125	240.460	1.982	8	8	100.000
1	9	5	12.643	9.571	46.560	25.286	40.286	12.000	27.643	2.429	381.155	14.667	287.143	10.286	7	7	100.000
1	9	6	10.778	7.333	30.819	27.250	28.750	12.500	17.625	4.625	108.929	18.000	64.768	28.839	9	8	88.889
1	9	7	10.571	6.643	33.369	7.060	33.929	11.286	23.357	4.643	261.619	16.571	280.893	5.226	7	7	100.000
1	9	8	9.688	7.000	11.067	5.143	37.563	11.875	27.875	4.875	161.746	4.696	129.554	0.696	8	8	100.000
1	9	9	10.409	8.636	10.691	6.655	46.545	13.273	36.136	4.636	277.873	7.418	216.805	3.655	11	11	100.000
1	9	10	9.545	7.455	7.073	3.073	33.500	12.455	23.955	5.000	250.800	10.073	226.873	5.000	11	11	100.000
1	9	11	9.455	7.545	8.023	6.873	35.773	12.364	26.318	4.818	181.068	5.855	154.464	1.564	11	11	100.000
1	9	12	18.750	9.500	0.125	24.500	47.000	14.000	28.250	4.500	1568.000	32.000	1540.125	0.500	2	2	100.000
1	9	13	12.750	11.000	45.125	32.000	81.250	16.500	68.500	5.500	15.125	24.500	8.000	0.500	2	2	100.000
1	9	14	16.000	18.000	*	*	77.500	19.000	61.500	1.000	*	*	*	*	1	1	100.000
1	9	15	13.500	5.500	60.500	4.500	59.000	11.000	45.500	5.500	364.500	8.000	128.000	0.500	2	2	100.000
1	9	16	12.625	8.750	20.563	7.583	51.875	15.000	39.250	6.250	248.063	22.000	217.750	4.250	4	4	100.000
1	9	17	27.750	10.000	903.125	72.000	53.000	13.500	25.250	3.500	32.000	40.500	595.125	4.500	2	2	100.000
1	9	18	22.000	19.000	*	*	81.500	26.000	59.500	7.000	*	*	*	*	1	1	100.000
1	10	1	10.500	12.000	*	*	72.000	19.000	61.500	7.000	*	*	*	*	1	1	100.000
1	10	2	9.400	8.800	17.675	16.700	26.300	12.600	16.900	3.800	17.325	2.300	14.550	7.700	5	5	100.000
1	10	3	10.250	7.875	3.929	6.696	48.938	13.875	38.688	6.000	266.531	16.411	239.781	7.143	8	8	100.000
1	10	4	10.563	9.625	14.603	17.696	45.063	14.500	34.500	4.875	227.388	19.714	163.000	3.268	8	8	100.000
1	10	5	10.286	9.143	3.905	6.810	42.071	13.286	31.786	4.143	389.786	6.905	339.655	1.476	7	7	100.000
1	10	6	12.350	7.200	50.725	5.289	35.900	11.200	23.550	4.000	222.322	5.289	243.192	2.667	10	10	100.000
1	10	7	14.857	8.286	178.393	19.238	39.786	13.143	24.929	4.857	244.238	6.476	264.369	9.810	7	7	100.000
1	10	8	11.438	8.000	16.888	8.286	32.000	11.250	20.563	3.250	306.500	8.214	242.103	2.786	8	8	100.000
1	10	9	10.409	8.636	9.041	18.855	51.091	14.000	40.682	5.364	549.091	19.000	497.264	2.255	11	11	100.000
1	10	10	9.000	6.636	8.000	8.055	38.818	11.091	29.818	4.455	365.964	17.091	291.014	3.473	11	11	100.000
1	10	11	9.200	9.000	7.956	22.667	39.950	12.600	30.750	3.600	345.192	21.156	309.069	6.267	10	10	100.000
1	10	12	17.250	14.500	210.125	84.500	87.750	19.500	70.500	5.000	231.125	40.500	0.500	8.000	2	2	100.000
1	10	13	24.750	11.000	91.125	32.000	84.500	18.500	59.750	7.500	1860.500	12.500	1128.125	84.500	2	2	100.000
1	10	14	8.000	6.000	*	*	29.000	8.000	21.000	2.000	*	*	*	*	1	1	100.000
1	10	15	18.750	17.000	66.125	98.000	75.500	19.000	56.750	2.000	840.500	32.000	435.125	18.000	2	2	100.000
1	10	16	12.500	14.333	7.750	6.333	73.667	18.667	61.167	4.333	162.333	2.333	102.083	16.333	3	3	100.000
1	10	17	57.500	15.500	8.000	4.500	92.250	21.000	34.750	5.500	780.125	18.000	630.125	4.500	2	2	100.000
1	10	18	9.500	9.500	4.500	12.500	45.000	13.500	35.500	4.000	612.500	12.500	512.000	0.000	2	2	100.000
1	11	1	12.000	8.000	*	*	30.500	14.000	18.500	6.000	*	*	*	*	1	1	100.000
1	11	2	9.500	8.000	11.500	9.500	35.300	12.400	25.800	4.400	286.700	15.300	215.700	2.300	5	5	100.000
1	11	3	8.357	6.857	2.476	5.143	33.500	11.857	25.143	5.000	74.083	3.476	58.310	2.667	7	7	100.000
1	11	4	9.125	7.875	9.911	11.554	41.563	12.375	32.438	4.500	342.603	9.411	299.174	5.429	8	8	100.000
1	11	5	11.214	9.286	8.071	39.571	43.417	13.667	32.167	3.833	620.342	19.067	525.767	10.167	7	6	85.714
1	11	6	11.500	9.500	34.000	22.722	41.900	13.300	30.400	3.800	440.044	10.900	411.489	6.178	10	10	100.000
1	11	7	9.929	7.929	5.536	4.036	32.857	11.857	22.929	3.929	180.310	7.810	152.202	1.702	7	7	100.000
1	11	8	9.188	6.375	14.781	4.268	30.375	10.000	21.188	3.625	362.482	9.714	251.781	3.411	8	8	100.000
1	11	9	13.273	10.636	20.118	11.855	46.500	15.455	33.227	4.818	346.300	12.873	286.668	9.364	11	11	100.000

Sites: 1= Oxon, 2 = Gloucestershire , 3 = Somerset. Prov = provenance, 98 and 99 = years, H = height (cm), D = stem diameter (mm), IH = height increment (cm), ID = stem diameter increment (mm), var = variance, N = number of trees per plot, Surv = survival %.

Site	Block	Prov	meanH98	meanD98	varH98	varD98	meanH99	meanD99	meanIH99	meanID99	varH99	varD99	varIH99	varID99	N98	N99	Surv98-99
1	11	10	11.818	8.455	25.364	7.273	39.909	12.636	28.091	4.182	387.391	7.455	364.291	4.164	11	11	100.000
1	11	11	8.182	7.273	7.364	7.018	34.778	11.889	26.222	4.111	373.194	1.611	395.944	4.361	11	9	81.818
1	11	12	12.250	10.000	36.125	50.000	53.000	15.500	40.750	5.500	1058.000	112.500	703.125	12.500	2	2	100.000
1	11	13	9.750	8.500	21.125	4.500	52.500	12.000	42.750	3.500	364.500	2.000	210.125	0.500	2	2	100.000
1	11	14	13.500	8.000	*	*	37.500	12.000	24.000	4.000	*	*	*	*	1	1	100.000
1	11	15	14.750	10.500	3.125	0.500	62.750	16.500	48.000	6.000	595.125	4.500	512.000	2.000	2	2	100.000
1	11	16	20.500	9.000	192.250	13.000	74.500	18.000	54.000	9.000	417.250	4.000	43.750	21.000	3	3	100.000
1	11	17	13.250	13.500	78.125	112.500	39.250	16.500	26.000	3.000	741.125	84.500	338.000	2.000	2	2	100.000
1	11	18	17.750	12.000	378.125	98.000	53.250	17.500	35.500	5.500	2628.125	112.500	1012.500	0.500	2	2	100.000
1	12	1	5.000	1.000	*	*	*	*	*	*	*	*	*	*	1	0	0.000
1	12	2	10.800	9.600	1.825	8.300	48.900	15.000	38.100	5.400	261.425	17.500	228.925	4.300	5	5	100.000
1	12	3	10.071	9.286	4.869	12.238	41.786	12.857	31.714	3.571	479.071	17.143	421.321	3.952	7	7	100.000
1	12	4	13.063	9.500	48.388	15.714	47.438	14.125	34.375	4.625	284.746	6.696	258.125	16.839	8	8	100.000
1	12	5	10.429	7.286	7.869	17.571	37.643	13.000	27.214	5.714	295.393	17.667	229.738	15.571	7	7	100.000
1	12	6	11.556	8.889	9.590	6.361	46.000	13.556	34.444	4.667	461.250	9.028	486.903	2.500	9	9	100.000
1	12	7	10.786	8.429	13.821	8.952	41.143	13.000	30.357	4.571	317.810	11.667	258.810	1.619	7	7	100.000
1	12	8	8.125	6.625	10.768	3.696	30.938	11.375	22.813	4.750	188.746	10.554	131.496	2.500	8	8	100.000
1	12	9	14.400	8.700	54.433	7.122	48.300	12.800	33.900	4.100	457.122	15.511	383.878	5.211	10	10	100.000
1	12	10	9.750	8.583	7.250	8.265	41.333	12.083	31.583	3.500	241.288	8.992	189.265	2.455	12	12	100.000
1	12	11	10.545	9.818	17.723	24.564	53.955	16.091	43.409	6.273	332.973	19.491	228.291	17.418	11	11	100.000
1	12	12	12.250	8.500	1.125	0.500	71.000	12.000	58.750	3.500	288.000	0.000	253.125	0.500	2	2	100.000
1	12	13	13.250	16.500	66.125	24.500	90.750	18.000	77.500	1.500	15.125	8.000	18.000	4.500	2	2	100.000
1	12	14	13.500	10.000	*	*	75.000	20.000	61.500	10.000	*	*	*	*	1	1	100.000
1	12	15	11.833	10.000	7.583	1.000	60.500	16.000	48.667	6.000	259.000	7.000	182.583	3.000	3	3	100.000
1	12	16	16.667	12.667	9.333	6.333	77.333	17.667	60.667	5.000	46.333	2.333	20.333	3.000	3	3	100.000
1	12	17	10.000	10.500	0.000	12.500	48.500	14.500	38.500	4.000	1740.500	4.500	1740.500	2.000	2	2	100.000
1	12	18	12.500	11.500	4.500	40.500	66.750	19.500	54.250	8.000	300.125	4.500	378.125	72.000	2	2	100.000
1	13	1	11.000	9.000	*	*	24.500	10.000	13.500	1.000	*	*	*	*	1	1	100.000
1	13	2	7.300	8.800	2.950	22.700	31.500	11.400	24.200	2.600	194.375	26.800	193.325	6.300	5	5	100.000
1	13	3	9.286	9.286	6.571	22.238	41.643	13.000	32.357	3.714	91.226	13.667	76.976	2.238	7	7	100.000
1	13	4	9.750	11.000	9.429	16.571	38.313	14.000	28.563	3.000	398.567	30.571	314.174	22.571	8	8	100.000
1	13	5	12.429	10.857	34.202	8.810	37.429	13.714	25.000	2.857	191.452	15.905	137.417	3.143	7	7	100.000
1	13	6	14.611	9.000	210.236	2.000	31.500	11.111	16.889	2.111	111.563	5.361	128.799	2.861	9	9	100.000
1	13	7	10.071	9.714	16.119	38.571	33.571	13.000	23.500	3.286	434.286	26.333	294.167	3.238	7	7	100.000
1	13	8	7.250	7.125	5.500	7.268	23.429	9.857	16.571	3.429	132.452	5.143	114.202	1.286	8	7	87.500
1	13	9	9.773	8.182	20.168	8.564	29.227	11.364	19.455	3.182	349.768	22.055	322.923	7.364	11	11	100.000
1	13	10	8.182	9.000	7.664	5.200	23.409	10.727	15.227	1.727	109.641	7.018	80.118	2.418	11	11	100.000
1	13	11	8.600	8.100	7.378	4.989	31.333	11.222	22.778	2.889	49.188	6.194	76.444	1.861	10	9	90.000
1	13	12	7.250	8.000	10.125	2.000	37.500	10.500	30.250	2.500	760.500	4.500	595.125	12.500	2	2	100.000
1	13	13	11.000	13.500	32.000	24.500	70.250	19.000	59.250	5.500	378.125	32.000	190.125	0.500	2	2	100.000
1	13	14	11.000	15.000	*	*	56.000	16.000	45.000	1.000	*	*	*	*	1	1	100.000
1	13	15	13.167	16.333	20.333	56.333	52.667	19.000	39.500	2.667	872.333	43.000	628.000	65.333	3	3	100.000
1	13	16	10.125	11.500	1.063	9.667	51.750	15.500	41.625	4.000	69.417	6.333	76.229	2.000	4	4	100.000
1	13	17	46.250	15.500	3240.125	112.500	56.500	20.500	10.250	5.000	2380.500	180.500	66.125	8.000	2	2	100.000
1	13	18	8.000	9.500	32.000	24.500	50.000	13.000	42.000	3.500	2312.000	72.000	1800.000	12.500	2	2	100.000
1	14	1	8.500	5.000	*	*	20.000	7.000	11.500	2.000	*	*	*	*	1	1	100.000
1	14	2	9.500	10.600	6.500	4.300	31.200	13.400	21.700	2.800	73.325	3.800	39.575	1.700	5	5	100.000
1	14	3	9.214	8.143	3.488	2.476	32.714	12.000	23.500	3.857	234.655	5.667	225.750	2.476	7	7	100.000
1	14	4	5.813	6.125	5.781	5.839	24.188	9.375	18.375	3.250	269.638	10.839	230.554	4.214	8	8	100.000
1	14	5	9.000	8.143	7.333	2.476	29.214	12.714	20.214	4.571	35.488	1.905	34.071	3.619	7	7	100.000
1	14	6	8.556	8.444	10.278	7.778	25.667	11.333	17.111	2.889	68.000	8.750	48.986	1.361	9	9	100.000
1	14	7	10.071	6.714	109.786	5.571	21.167	9.333	10.750	3.000	125.067	13.867	44.975	2.000	7	6	85.714
1	14	8	10.500	8.625	32.500	11.982	27.625	12.250	17.125	3.625	106.911	14.786	68.196	1.982	8	8	100.000
1	14	9	8.550	7.600	7.692	5.822	30.150	11.300	21.600	3.700	94.725	6.233	81.267	1.567	10	10	100.000
1	14	10	9.182	7.909	8.014	7.491	30.091	11.455	20.909	3.545	161.491	17.273	123.241	4.273	11	11	100.000
1	14	11	8.409	8.364	13.541	10.655	25.000	11.000	16.591	2.636	151.600	15.000	92.391	2.855	11	11	100.000
1	14	12	18.750	18.000	10.125	18.000	77.000	24.500	58.250	6.500	18.000	12.500	55.125	0.500	2	2	100.000
1	14	13	9.250	8.000	6.125	8.000	42.000	11.000	32.750	3.000	162.000	2.000	105.125	2.000	2	2	100.000
1	14	14	26.000	23.000	*	*	74.000	23.000	48.000	0.000	*	*	*	*	1	1	100.000
1	14	15	8.250	8.500	3.125	12.500	50.000	13.000	41.750	4.500	2.000	8.000	0.125	0.500	2	2	100.000
1	14	16	15.250	10.750	132.917	24.917	47.375	14.750	32.125	4.000	207.063	24.917	65.396	2.667	4	4	100.000
1	14	17	10.500	11.500	18.000	60.500	41.250	13.500	30.750	2.000	1485.125	60.500	1176.125	0.000	2	2	100.000
1	14	18	4.000	3.000	0.500	0.000	11.500	5.500	7.500	2.500	4.500	0.500	2.000	0.500	2	2	100.000
1	14	19	11.000	13.000	*	*	38.500	15.000	27.500	2.000	*	*	*	*	1	1	100.000
2	15	1	11.000	8.000	*	*	44.000	16.000	33.000	8.000	*	*	*	*	1	1	100.000
2	15	2	10.167	7.333	4.083	5.333	34.333	12.667	24.167	5.333	88.083	2.333	121.333	2.333	3	3	100.000
2	15	3	10.750	11.000	4.175	20.000	39.917	16.000	29.167	5.000	418.342	13.600	412.267	8.400	6	6	100.000
2	15	4	8.917	7.833	4.842	2.967	31.417	12.333	22.500	4.500	59.542	5.467	60.800	3.500	6	6	100.000
2	15	5	8.500	7.600	7.375	3.300	28.600	12.600	20.100	5.000	245.425	7.300	268.675	3.500	5	5	100.000
2	15	6	10.667	9.500	6.767	11.900	29.333	12.833	18.667	3.333	18.267	11.767	9.267	0.667	6	6	100.000
2	15	7	9.857	8.714	8.810	8.571	48.071	13.714	38.214	5.000	288.119	16.905	225.821	4.000	7	7	100.000
2	15	8	10.143	8.714	2.310	11.238	27.929	13.286	17.786	4.571	89.202	23.571	84.821	4.286	7	7	100.000
2	15	9	12.800	9.000	4.575	10.000	46.900	13.600	34.100	4.600	514.675	12.800	491.300	7.300	5	5	100.000
2	15	10	11.357	10.143	6.226	5.476	35.429	15.429	24.071	5.286	142.369	5.619	122.369	12.905	7	7	100.000
2	15	11	10.100	11.000	1.300	1.000	38.400	14.800	28.300	3.800	193.800	2.200	168.325	4.700	5	5	100.000
2	15	12	17.500	12.000	0.500	2.000	60.500	18.000	43.000	6.000	12.500	8.000	8.000	2.000	2	2	100.000
2	15	13	13.125	12.500	41.229	7.000	69.250	14.750	56.125	2.250	216.917	7.583	74.229	2.917	4	4	100.000
2	15	14	13.167	15.667	13.083	16.333	54.333	18.667	41.167	3.000	277.083	6.333	245.583	3.000	3	3	100.000
2	15	15	11.625	10.750	21.229	20.250	49.375	15.000	37.750	4.250	197.063	14.667	224.417	4.917	4	4	100.000
2	15	16	7.786	6.857	14.738	15.810	32.714	12.571	24.929	5.714	346.571	28.952	226.702	30.571	7	7	100.000
2	15	17	15.333	12.667	6.333	10.333	52.833	16.000	37.500	3.333	79.083	7.000	42.250	1.333	3	3	100.000
2	15	18	25.250	22.000	0.125	0.000	56.750	24.000	31.500	2.000	21.125	2.000	24.500	2.000	2	2	100.000
2	15	19	8.500	9.000	*	*	32.000	13.000	23.500	4.000	*	*	*	*	1	1	100.000
2	15	20	6.500	5.000	*	*	16.500	8.000	10.000	3.000	*	*	*	*	1	1	100.000
2	15	21	34.800	16.000	57.075	5.500	65.700	20.200	30.900	4.200	165.700	21.700	155.300	12.200	5	5	100.000
2	15	22	12.333	8.333	17.333	17.333	40.500	13.000	28.167	4.667	204.750	9.000	105.083	2.333	3	3	100.000
2	15	23	15.500	13.000	4.500	2.000	51.000	17.000	35.500	4.000	2.000	8.000	0.500	2.000	2	2	100.000
2	15	24	55.167	21.000	553.083	39.000	73.167	24.000	18.000	3.000	456.333	61.000	263.250	4.000	3	3	100.000
2	15	25	12.000	11.000	84.500	98.000	49.250	14.000	37.250	3.000	3081.125	98.000	2145.125	0.000	2	2	100.000
2	16	1	4.500	3.000	*	*	12.000	6.000	7.500	3.000	*	*	*	*	1	1	100.000
2	16	2	13.167	10.667	2.583	4.333	51.833	16.333	38.667	5.667	232.583	10.333	281.083	2.333	3	3	100.000
2	16	3	9.600	6.800	2.425	2.700	32.000	12.800	22.400	6.000	196.875	10.700	168.925	4.000	5	5	100.000
2	16	4	15.667	9.833	197.867	18.967	44.917	14.167	29.250	4.333	232.842	9.367	225.175	3.067	6	6	100.000
2	16	5	16.083	8.833	73.442	21.767	34.333	14.167	18.250	5.333	186.167	12.967	74.875	4.267	6	6	100.000
2	16	6	13.667	7.500	31.767	3.500	33.667	11.667	20.000	4.167	152.367	2.667	216.800	5.767	6	6	100.000

Sites: 1= Oxon, 2 = Gloucestershire , 3 = Somerset. Prov = provenance, 98 and 99 = years, H = height (cm), D = stem diameter (mm), IH = height increment (cm), ID = stem diameter increment (mm), var = variance, N = number of trees per plot, Surv = survival %.

Site	Block	Prov	meanH98	meanD98	varH98	varD98	meanH99	meanD99	meanIH99	meanID99	varH99	varD99	varIH99	varID99	N98	N99	Surv98-99
2	16	7	11.857	9.571	70.560	48.619	38.571	13.857	26.714	4.286	483.202	45.143	218.155	32.571	7	7	100.000
2	16	8	9.929	8.286	13.536	13.238	37.143	12.571	27.214	4.286	206.810	4.952	140.905	8.571	7	7	100.000
2	16	9	9.800	9.800	9.200	3.200	36.100	16.200	26.300	6.400	251.425	4.700	212.325	1.300	5	5	100.000
2	16	10	12.429	10.714	1.286	4.905	44.071	15.857	31.643	5.143	170.119	12.810	164.476	3.476	7	7	100.000
2	16	11	11.700	9.400	14.450	14.300	38.900	14.200	27.200	4.800	207.300	12.700	162.700	11.200	5	5	100.000
2	16	12	23.000	13.500	8.000	0.500	69.000	20.000	46.000	6.500	480.500	8.000	364.500	4.500	2	2	100.000
2	16	13	10.875	11.000	29.063	30.000	50.500	16.500	39.625	5.500	1500.167	73.000	1208.729	15.000	4	4	100.000
2	16	14	13.167	12.667	71.583	14.333	53.667	15.000	40.500	2.333	595.083	12.000	366.750	0.333	3	3	100.000
2	16	15	11.125	10.000	20.563	22.000	49.500	15.750	38.375	5.750	504.667	29.583	339.229	2.250	4	4	100.000
2	16	16	13.643	10.571	9.060	7.619	50.500	16.143	36.857	5.571	402.000	8.810	311.476	3.286	7	7	100.000
2	16	17	24.000	12.667	417.000	54.333	56.000	18.333	32.000	5.667	364.000	104.333	31.000	8.333	3	3	100.000
2	16	18	10.500	9.000	24.500	32.000	31.000	10.500	20.500	1.500	512.000	40.500	312.500	0.500	2	2	100.000
2	16	19	9.000	4.000	*	*	25.000	11.000	16.000	7.000	*	*	*	*	1	1	100.000
2	16	20	16.000	14.000	*	*	74.500	17.000	58.500	3.000	*	*	*	*	1	1	100.000
2	16	21	39.600	15.200	98.300	11.700	66.500	21.400	26.900	6.200	253.000	32.800	127.300	8.200	5	5	100.000
2	16	22	9.500	7.667	1.750	2.333	39.000	12.667	29.500	5.000	95.250	2.333	72.250	0.000	3	3	100.000
2	16	23	16.500	11.500	60.500	0.500	66.750	16.500	50.250	5.000	253.125	4.500	561.125	2.000	2	2	100.000
2	16	24	57.500	19.333	120.250	17.333	77.500	22.667	20.000	3.333	248.250	41.333	267.250	9.333	3	3	100.000
2	16	25	13.500	10.000	*	*	48.000	17.000	34.500	7.000	*	*	*	*	1	1	100.000
2	16	26	14.000	10.000	*	*	69.000	16.000	55.000	6.000	*	*	*	*	1	1	100.000
2	17	1	10.000	7.000	*	*	38.000	14.000	28.000	7.000	*	*	*	*	1	1	100.000
2	17	2	7.625	6.500	11.229	12.333	31.500	12.667	22.833	5.000	295.750	16.333	198.083	1.000	4	3	75.000
2	17	3	8.800	7.800	4.700	13.700	38.900	16.000	30.100	8.200	632.550	76.500	546.800	32.200	5	5	100.000
2	17	4	9.083	7.333	15.242	4.667	31.333	11.500	22.250	4.167	194.967	7.100	210.575	2.167	6	6	100.000
2	17	5	11.700	9.600	2.700	4.300	41.500	14.200	29.800	4.600	53.375	0.700	60.825	1.800	5	5	100.000
2	17	6	9.000	8.500	7.200	9.900	30.833	13.500	21.833	5.000	160.967	17.100	136.367	4.400	6	6	100.000
2	17	7	10.250	9.333	15.875	22.267	42.917	14.000	32.667	4.667	249.542	17.600	154.667	6.267	6	6	100.000
2	17	8	9.214	7.714	5.988	4.238	31.286	11.857	22.071	4.143	75.238	6.476	56.036	2.476	7	7	100.000
2	17	9	13.200	10.600	19.200	24.800	44.500	14.800	31.300	4.200	454.375	22.700	335.575	2.700	5	5	100.000
2	17	10	11.643	9.286	15.060	4.905	38.786	15.143	27.143	5.857	51.655	7.143	68.726	5.143	7	7	100.000
2	17	11	8.917	7.500	14.242	12.300	26.250	12.833	17.333	5.333	79.475	16.567	29.967	3.067	6	6	100.000
2	17	12	19.500	13.500	60.500	12.500	61.000	17.000	41.500	3.500	578.000	2.000	1012.500	4.500	2	2	100.000
2	17	13	11.250	10.000	23.583	19.333	55.625	17.000	44.375	7.000	1093.729	26.000	845.729	2.667	4	4	100.000
2	17	14	11.833	11.333	56.583	21.333	52.167	17.000	40.333	5.667	308.333	21.000	119.083	2.333	3	3	100.000
2	17	15	14.875	12.500	13.063	9.000	60.875	19.500	46.000	7.000	491.063	49.667	421.500	16.667	4	4	100.000
2	17	16	13.357	9.857	24.726	12.143	51.071	15.000	37.714	5.143	338.286	10.333	326.655	1.810	7	7	100.000
2	17	17	13.667	9.000	165.083	61.000	40.333	13.333	26.667	4.333	1635.583	102.333	763.083	5.333	3	3	100.000
2	17	18	16.000	14.500	18.000	24.500	61.000	20.000	45.000	5.500	24.500	32.000	0.500	0.500	2	2	100.000
2	17	19	10.000	7.000	*	*	50.500	13.000	40.500	6.000	*	*	*	*	1	1	100.000
2	17	20	12.000	8.000	*	*	36.500	12.000	24.500	4.000	*	*	*	*	1	1	100.000
2	17	21	39.600	13.600	72.675	9.300	62.000	19.600	22.400	6.000	315.625	20.800	101.050	8.500	5	5	100.000
2	17	22	11.333	10.333	4.333	4.333	42.833	13.667	31.500	3.333	150.583	1.333	106.750	2.333	3	3	100.000
2	17	23	17.000	9.000	162.000	18.000	47.000	15.500	30.000	6.500	840.500	12.500	264.500	0.500	2	2	100.000
2	17	24	49.333	22.333	66.333	16.333	79.833	28.333	30.500	6.000	14.083	9.333	30.250	7.000	3	3	100.000
2	17	25	12.000	8.500	8.000	4.500	18.750	16.000	6.750	7.500	1.125	72.000	15.125	40.500	2	2	100.000
2	18	1	3.500	2.000	*	*	37.000	16.000	33.500	14.000	*	*	*	*	1	1	100.000
2	18	2	6.875	5.000	10.063	2.000	21.375	10.250	14.500	5.250	295.563	20.250	212.833	12.250	4	4	100.000
2	18	3	8.917	6.667	11.442	1.867	27.833	11.500	18.917	4.833	92.867	1.900	155.042	1.367	6	6	100.000
2	18	4	8.667	6.667	3.367	3.867	37.750	12.500	29.083	5.833	155.675	4.300	141.542	0.567	6	6	100.000
2	18	5	10.300	8.200	4.200	0.700	28.400	13.400	18.100	5.200	32.800	4.300	39.550	3.200	5	5	100.000
2	18	6	12.417	7.500	52.642	3.500	37.583	14.167	25.167	6.667	137.842	6.567	189.667	1.867	6	6	100.000
2	18	7	9.667	8.500	5.767	5.500	38.250	14.167	28.583	5.667	150.175	11.367	139.942	3.867	6	6	100.000
2	18	8	10.786	6.857	34.071	2.810	32.143	12.571	21.357	5.714	153.810	4.286	143.226	2.238	7	7	100.000
2	18	9	11.583	8.000	33.442	11.200	35.583	13.500	24.000	5.500	251.542	15.500	178.600	4.300	6	6	100.000
2	18	10	9.833	8.500	2.167	5.500	36.000	13.500	26.167	5.000	50.000	7.500	50.967	1.200	6	6	100.000
2	18	11	10.500	6.800	0.500	3.700	32.300	11.000	21.800	4.200	323.200	8.500	321.575	2.700	5	5	100.000
2	18	12	13.750	9.500	66.125	12.500	63.500	15.000	49.750	5.500	84.500	2.000	1.125	4.500	2	2	100.000
2	18	13	12.625	12.250	2.563	26.250	74.375	18.000	61.750	5.750	109.229	20.667	78.417	4.917	4	4	100.000
2	18	14	12.000	11.667	31.000	14.333	39.333	15.333	27.333	3.667	456.083	20.333	252.583	2.333	3	3	100.000
2	18	15	10.000	7.750	30.000	26.917	44.500	14.750	34.500	7.000	404.500	49.583	279.500	6.000	4	4	100.000
2	18	16	10.929	7.571	25.369	13.286	47.286	13.714	36.357	6.143	614.905	22.238	487.060	3.476	7	7	100.000
2	18	17	7.833	4.667	2.583	5.333	24.000	7.333	16.167	2.667	193.000	8.333	152.083	0.333	3	3	100.000
2	18	18	9.500	7.500	60.500	40.500	37.500	11.500	28.000	4.000	242.000	40.500	60.500	0.000	2	2	100.000
2	18	19	8.000	6.000	*	*	41.000	17.000	33.000	11.000	*	*	*	*	1	1	100.000
2	18	20	14.000	6.000	*	*	51.000	11.000	37.000	5.000	*	*	*	*	1	1	100.000
2	18	21	51.900	15.200	159.050	4.700	76.600	20.000	24.700	4.800	48.425	1.500	112.325	4.200	5	5	100.000
2	18	22	19.333	8.667	52.333	1.333	25.833	9.667	6.500	1.000	214.083	20.333	399.250	31.000	3	3	100.000
2	18	23	22.500	7.000	312.500	8.000	41.250	13.000	18.750	6.000	21.125	32.000	171.125	8.000	2	2	100.000
2	18	24	44.333	19.333	345.333	30.333	67.833	25.333	23.500	6.000	604.333	112.333	37.000	39.000	3	3	100.000
2	18	25	13.000	11.000	98.000	2.000	65.000	15.500	52.000	4.500	242.000	0.500	32.000	0.500	2	2	100.000
3	19	1	10.000	13.000	*	*	27.000	18.000	17.000	5.000	*	*	*	*	1	1	100.000
3	19	2	10.667	14.667	1.583	34.333	35.833	16.667	25.167	2.000	91.583	1.333	115.583	27.000	3	3	100.000
3	19	3	10.583	9.333	9.542	7.067	35.417	16.500	24.833	7.167	199.142	10.300	180.567	1.767	6	6	100.000
3	19	4	11.857	10.714	18.726	6.571	39.214	16.857	27.357	6.143	113.655	12.476	117.393	13.143	7	7	100.000
3	19	5	10.500	7.600	8.500	4.300	24.200	11.600	13.700	4.000	134.200	5.800	97.200	5.500	5	5	100.000
3	19	6	11.917	11.000	8.542	7.600	38.917	17.833	27.000	6.833	233.042	14.167	270.200	6.167	6	6	100.000
3	19	7	8.750	9.167	22.175	15.767	29.000	14.500	20.250	5.333	157.100	27.100	91.575	8.667	6	6	100.000
3	19	8	11.286	11.286	1.738	15.238	41.071	18.286	29.786	7.000	54.119	26.238	46.905	22.333	7	7	100.000
3	19	9	12.583	13.167	7.342	6.167	61.833	20.833	49.250	7.667	410.267	8.567	457.075	13.467	6	6	100.000
3	19	10	19.333	8.500	235.467	8.700	29.500	14.167	10.167	5.667	211.800	12.967	215.567	15.467	6	6	100.000
3	19	11	12.700	10.400	4.825	0.300	41.800	18.000	29.100	7.600	211.325	4.500	226.300	5.800	5	5	100.000
3	19	12	11.750	11.500	6.125	0.500	85.000	20.000	73.250	8.500	40.500	18.000	15.125	12.500	2	2	100.000
3	19	13	17.875	15.750	132.729	24.917	85.250	25.250	67.375	9.500	129.083	26.917	143.396	19.000	4	4	100.000
3	19	14	10.667	9.000	7.583	9.000	59.500	17.000	48.833	8.000	687.250	25.000	793.083	19.000	3	3	100.000
3	19	15	9.750	8.000	17.083	4.667	44.250	16.250	34.500	8.250	511.417	7.583	407.167	3.583	4	4	100.000
3	19	16	12.000	9.857	69.333	40.476	52.643	15.429	40.643	5.571	1272.643	91.619	963.476	16.619	7	7	100.000
3	19	17	30.833	21.000	690.333	43.000	87.833	25.333	57.000	4.333	210.333	8.333	756.000	16.333	3	3	100.000
3	19	18	20.000	17.000	4.500	18.000	74.750	27.000	54.750	10.000	6.125	18.000	21.125	0.000	2	2	100.000
3	19	19	10.500	8.000	*	*	53.000	15.000	42.500	7.000	*	*	*	*	1	1	100.000
3	19	20	17.500	10.000	*	*	43.000	18.000	25.500	8.000	*	*	*	*	1	1	100.000
3	19	21	42.900	18.600	173.550	15.800	83.200	28.000	40.300	9.400	247.200	30.000	340.700	4.300	5	5	100.000
3	19	22	18.167	14.667	30.083	9.333	48.833	17.667	30.667	3.000	339.083	0.333	569.333	9.000	3	3	100.000
3	19	23	12.000	11.500	12.500	4.500	93.750	25.000	81.750	13.500	253.125	18.000	153.125	4.500	2	2	100.000
3	19	24	46.167	24.333	72.583	10.333	87.667	26.333	41.500	2.000	141.583	14.333	375.250	7.000	3	3	100.000
3	19	25	12.250	11.500	66.125	4.500	62.000	21.000	49.750	9.500	512.000	32.000	210.125	12.500	2	2	100.000

Sites: 1= Oxon, 2 = Gloucestershire , 3 = Somerset. Prov = provenance, 98 and 99 = years, H = height (cm), D = stem diameter (mm), IH = height increment (cm), ID = stem diameter increment (mm), var = variance, N = number of trees per plot, Surv = survival %.

Site	Block	Prov	meanH98	meanD98	varH98	varD98	meanH99	meanD99	meanIH99	meanID99	varH99	varD99	varIH99	varID99	N98	N99	Surv98-99
3	20	1	8.500	9.000	*	*	25.500	16.000	17.000	7.000	*	*	*	*	1	1	100.000
3	20	2	12.167	11.333	1.583	2.333	31.167	15.667	19.000	4.333	31.083	12.333	19.000	4.333	3	3	100.000
3	20	3	11.286	9.429	16.821	7.952	37.857	15.429	26.571	6.000	301.976	17.286	238.702	3.667	7	7	100.000
3	20	4	11.417	12.000	7.942	2.400	38.333	17.833	26.917	5.833	163.567	12.167	116.942	7.767	6	6	100.000
3	20	5	9.600	9.200	5.800	2.700	43.600	16.400	34.000	7.200	256.675	9.800	195.875	2.700	5	5	100.000
3	20	6	11.917	8.167	16.842	1.367	29.250	13.167	17.333	5.000	24.475	0.967	69.567	2.000	6	6	100.000
3	20	7	10.929	7.571	17.036	7.286	34.286	14.571	23.357	7.000	430.155	30.952	289.226	12.333	7	7	100.000
3	20	8	9.643	9.286	8.476	10.238	25.929	12.857	16.286	3.571	108.286	6.810	95.155	5.952	7	7	100.000
3	20	9	11.600	7.800	1.800	3.200	39.500	14.800	27.900	7.000	283.875	7.700	252.175	3.500	5	5	100.000
3	20	10	13.167	10.000	42.767	20.400	36.750	14.833	23.583	4.833	216.375	16.967	93.742	2.167	6	6	100.000
3	20	11	9.800	8.600	6.325	5.300	49.000	18.400	39.200	9.800	445.875	10.800	458.075	4.700	5	5	100.000
3	20	12	16.250	14.000	0.125	2.000	86.000	25.000	69.750	11.000	18.000	8.000	15.125	2.000	2	2	100.000
3	20	13	13.625	13.250	10.229	10.250	96.875	26.000	83.250	12.750	550.729	95.333	470.917	48.917	4	4	100.000
3	20	14	19.833	14.333	43.583	9.333	77.000	22.333	57.167	8.000	247.000	10.333	475.083	7.000	3	3	100.000
3	20	15	13.500	12.500	10.500	7.000	68.250	22.500	54.750	10.000	408.250	11.000	415.750	18.000	4	4	100.000
3	20	16	16.857	10.857	128.893	3.810	62.857	17.000	46.000	6.143	257.976	4.667	257.667	3.143	7	7	100.000
3	20	17	15.167	11.667	23.583	0.333	85.500	20.667	70.333	9.000	111.000	8.333	236.083	7.000	3	3	100.000
3	20	18	8.750	10.500	55.125	40.500	38.500	15.000	29.750	4.500	924.500	98.000	528.125	12.500	2	2	100.000
3	20	19	10.000	7.000	*	*	30.000	15.000	20.000	8.000	*	*	*	*	1	1	100.000
3	20	20	19.000	17.000	*	*	108.500	35.000	89.500	18.000	*	*	*	*	1	1	100.000
3	20	21	35.300	16.000	68.200	12.500	75.300	25.600	40.000	9.600	155.950	30.800	42.500	24.800	5	5	100.000
3	20	22	17.167	11.667	14.083	10.333	70.667	18.667	53.500	7.000	77.083	21.333	150.750	21.000	3	3	100.000
3	20	23	10.500	12.500	0.500	0.500	77.750	21.000	67.250	8.500	276.125	8.000	300.125	4.500	2	2	100.000
3	20	24	43.500	16.333	82.750	14.333	87.500	23.000	44.000	6.667	316.750	28.000	126.750	4.333	3	3	100.000
3	20	25	12.550	12.500	70.805	24.500	58.750	19.500	46.200	7.000	2850.125	180.500	2022.480	72.000	2	2	100.000
3	21	1	9.500	10.000	*	*	38.000	17.000	28.500	7.000	*	*	*	*	1	1	100.000
3	21	2	9.667	10.333	1.333	5.333	34.333	17.000	24.667	6.667	83.083	9.000	68.083	14.333	3	3	100.000
3	21	3	10.333	9.167	18.767	8.967	37.833	16.000	27.500	6.833	610.067	17.600	470.600	4.567	6	6	100.000
3	21	4	13.333	11.000	7.767	10.400	39.750	19.500	26.417	8.500	368.075	31.100	449.042	36.300	6	6	100.000
3	21	5	10.700	8.200	11.325	4.200	27.500	14.200	16.800	6.000	23.500	5.700	18.325	3.500	5	5	100.000
3	21	6	14.667	9.833	31.767	4.567	36.000	15.167	21.333	5.333	142.300	5.767	150.167	2.667	6	6	100.000
3	21	7	11.857	9.000	12.393	3.333	41.143	17.143	29.286	8.143	523.060	28.476	531.071	24.476	7	7	100.000
3	21	8	10.750	8.500	2.075	2.700	35.333	16.500	24.583	8.000	102.367	9.500	88.542	8.000	6	6	100.000
3	21	9	13.300	9.400	15.200	11.300	42.400	18.600	29.100	9.200	196.925	32.300	134.925	24.200	5	5	100.000
3	21	10	12.357	10.000	3.560	5.333	35.786	16.857	23.429	6.857	72.071	11.476	85.536	5.810	7	7	100.000
3	21	11	12.917	8.333	20.142	6.267	29.917	12.833	17.000	4.500	50.542	3.767	31.300	1.100	6	6	100.000
3	21	12	14.500	11.500	4.500	0.500	56.500	16.500	42.000	5.000	1682.000	4.500	1512.500	8.000	2	2	100.000
3	21	13	16.500	11.500	42.167	30.333	53.125	19.000	36.625	7.500	589.063	58.000	454.396	9.667	4	4	100.000
3	21	14	10.333	11.000	1.083	13.000	78.500	22.333	68.167	11.333	169.750	34.333	150.583	5.333	3	3	100.000
3	21	15	21.875	12.750	155.563	6.917	83.250	25.250	61.375	12.500	249.750	18.917	386.063	33.667	4	4	100.000
3	21	16	20.571	12.857	65.619	13.143	71.929	22.429	51.357	9.571	436.452	39.619	401.976	14.286	7	7	100.000
3	21	17	36.750	14.500	903.125	12.500	90.750	30.500	54.000	16.000	10.125	4.500	722.000	2.000	2	2	100.000
3	21	18	13.500	10.000	50.000	0.000	52.500	22.000	39.000	12.000	2048.000	98.000	1458.000	98.000	2	2	100.000
3	21	19	12.000	9.000	*	*	90.000	19.000	78.000	10.000	*	*	*	*	1	1	100.000
3	21	20	11.000	9.000	*	*	77.000	25.000	66.000	16.000	*	*	*	*	1	1	100.000
3	21	21	32.700	14.000	197.325	10.000	43.900	18.200	11.200	4.200	457.300	11.700	70.450	1.700	5	5	100.000
3	21	22	28.000	14.000	612.250	13.000	87.000	20.667	59.000	6.667	361.000	32.333	194.250	4.333	3	3	100.000
3	21	23	15.000	12.000	8.000	2.000	99.250	22.500	84.250	10.500	136.125	0.500	78.125	4.500	2	2	100.000
3	21	24	47.000	16.667	343.000	10.333	68.000	23.667	21.000	7.000	379.750	46.333	85.750	16.000	3	3	100.000
3	21	25	12.833	9.000	39.083	4.000	73.000	17.667	60.167	8.667	27.000	32.333	27.083	14.333	3	3	100.000
3	22	1	11.500	9.000	*	*	30.000	14.000	18.500	5.000	*	*	*	*	1	1	100.000
3	22	2	9.750	9.000	0.125	2.000	25.500	14.500	15.750	5.500	12.500	0.500	10.125	4.500	2	2	100.000
3	22	3	13.200	9.800	8.200	3.700	28.400	14.600	15.200	4.800	86.425	12.300	63.450	6.700	5	5	100.000
3	22	4	11.917	9.333	7.342	3.067	33.750	16.667	21.833	7.333	217.375	16.667	190.467	9.867	6	6	100.000
3	22	5	11.750	9.333	6.675	0.667	42.833	15.667	31.083	6.333	58.267	4.667	66.642	4.267	6	6	100.000
3	22	6	11.333	9.333	5.967	9.467	28.083	14.167	16.750	4.833	112.442	11.767	120.275	1.767	6	6	100.000
3	22	7	11.357	8.143	3.310	0.476	35.071	15.143	23.714	7.000	393.619	8.143	353.238	6.000	7	7	100.000
3	22	8	10.286	9.714	1.905	6.571	31.286	15.571	21.000	5.857	136.905	9.952	140.833	23.810	7	7	100.000
3	22	9	12.500	9.143	14.333	6.810	31.143	12.857	18.643	3.714	280.726	23.476	279.976	8.238	7	7	100.000
3	22	10	10.583	9.833	9.942	10.567	38.800	17.200	27.300	6.200	706.825	24.700	600.825	13.700	6	5	83.333
3	22	11	11.083	8.500	3.642	8.300	28.800	14.400	17.500	6.600	88.075	17.800	83.125	5.800	6	5	83.333
3	22	12	11.250	8.500	28.125	12.500	68.500	18.000	57.250	9.500	800.000	8.000	528.125	0.500	2	2	100.000
3	22	13	8.000	9.250	5.833	14.917	45.000	13.250	37.000	4.000	1078.167	18.250	931.333	11.333	4	4	100.000
3	22	14	17.667	15.000	52.333	28.000	53.500	20.667	35.833	5.667	545.250	30.333	281.583	16.333	3	3	100.000
3	22	15	13.500	7.000	41.667	8.667	37.750	12.250	24.250	5.250	911.583	48.917	904.917	18.250	4	4	100.000
3	22	16	18.143	12.429	111.226	13.619	54.929	18.571	36.786	6.143	578.452	22.952	434.071	12.810	7	7	100.000
3	22	17	14.667	12.000	5.333	3.000	69.333	21.000	54.667	9.000	33.583	28.000	65.583	43.000	3	3	100.000
3	22	18	12.500	13.000	60.500	32.000	31.000	16.000	18.500	3.000	512.000	72.000	220.500	8.000	2	2	100.000
3	22	19	18.500	8.000	*	*	72.000	17.000	53.500	9.000	*	*	*	*	1	1	100.000
3	22	20	6.000	5.000	*	*	22.000	11.000	16.000	6.000	*	*	*	*	1	1	100.000
3	22	21	37.800	17.000	75.700	11.500	73.700	24.000	35.900	7.000	1078.075	28.000	639.175	6.500	5	5	100.000
3	22	22	18.000	11.667	93.000	1.333	78.167	19.667	60.167	8.000	490.083	4.333	637.583	7.000	3	3	100.000
3	22	23	10.500	12.000	*	*	74.000	14.000	63.500	2.000	*	*	*	*	1	1	100.000
3	22	24	32.833	19.667	196.083	6.333	77.333	28.000	44.500	8.333	623.583	3.000	798.250	2.333	3	3	100.000
3	22	25	20.250	12.000	91.125	18.000	55.500	18.500	35.250	6.500	1512.500	24.500	861.125	0.500	2	2	100.000

Sites: 1= Oxon, 2 = Gloucestershire , 3 = Somerset. Prov = provenance, 98 and 99 = years, H = height (cm), D = stem diameter (mm), IH = height increment (cm), ID = stem diameter increment (mm), var = variance, N = number of trees per plot, Surv = survival %.