GENETIC VARIATION IN EARLY GROWTH AND IN PRODUCTIVITY UNDER WATER STRESS IN *GREVILLEA ROBUSTA* A. CUNN

by

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Statement of originality of the thesis

Except where specific acknowledgments are given, this thesis is my own original work.

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ABSTRACT

Grevillea robusta A. Cunn. Ex R. Br. is native to the Australia's east coastal subtropical forests. Although not presently of economic importance in Australia, the species is very popular as a multipurpose tree and is widely planted in many countries for provision of a wide range of products and services. It is one of the most important agroforestry tree species component in many tropical countries and its traditional uses as an ornamental and as shade in tea and coffee are well known.

A study of genetic variation using natural seed sources of the species was carried out to provide information that may be needed for selection and improvement of the species. The influence of water stress on growth performance of the species was also studied to understand guidelines for use of the species in marginal conditions.

Eleven provenances of *G. robusta* with three families in each were grown under semi-controlled glasshouse conditions and assessed for growth characteristics. Results showed evidence of genetic variation between provenances and between families within provenances. Most of the variation was distributed between provenances, with a smaller, though not negligible magnitude between families within provenances. A number of basic traits such as height, diameter and leaf area showed clinal patterns of variation, with altitudinal clines being prominent. Provenances from high altitudes of seed source had slower growth compared to low altitude coastal sources. Temperature of seed source was important in explaining altitudinal variation in the species.

Principal Component Analysis showed that the lower altitude coastal provenances of *G. robusta* segregated together, whilst the middle and high altitude provenances formed a second, more divergent group. The two major groups corresponded to the riverine and dry upland habitat types that characterise the species' area of natural distribution. Most traits showed high heritability values and significant correlations among seedling traits.

The effect of water stress on growth and productivity of five provenances of *G. robusta* derived from the riverine and dry upland sources also were studied. Water stress caused reduction in growth with leaf area showing most sensitivity. Among traits assessed, the drought avoidance characteristics of leaf area reduction and slow growth were identified as important in coping with water stress in seedlings of *G. robusta*. Sources from the wet and dry upland habitat types differed in response to water stress, with the dry habitat sources likely to be more adapted to drought stress than the riverine sources. Variation between provenance and families within provenance in response to water stress was also evident. It may be possible to select the dry upland sources of *G. robusta* for planting in semi arid conditions and to use the riverine sources for more favourable environmental conditions.

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CHAPTER 1

INTRODUCTION

1.1 The importance of multipurpose trees

Much of the wood produced in the world is used by communities in tropical and sub-tropical developing countries. It has been estimated that over half of the wood produced throughout the world each year is used up by such countries as fuelwood and charcoal for cooking and heating (Campinhos, 1991).

In many of these countries, plantation forestry became important in the late 1960's due to a high demand for wood and other forest products. In the late 1970's, tree planting underwent a change, with more emphasis placed on use of genetically and physiologically better planting material (Simmons, 1992). At the same time, the rapidly increasing demand for forest products led to a gradual decline of natural woody vegetation.

The bulk of the wood produced from plantation forests does not directly benefit many rural communities, at least in terms of their immediate needs because it is sold directly to factories and industries for processing. As a counter to this, the emerging pattern of reforestation especially in developing countries is of equal, if not more, emphasis on community based forestry and agroforestry than on industrial plantation forestry. While large scale plantation forestry will probably continue to expand to meet demands for wood, it is likely to be increasingly complemented by other forms of tree growing more closely integrated with other forms of land-use (Kanowski and Savill, 1992).

Planting of multi-purpose trees has thus become increasingly important in many rural development schemes in most developing tropical and sub-tropical countries as a supplement to other forms of tree planting. Multipurpose trees provide a wide range of products including fuelwood, building materials, fodder shade and environmental benefits such as erosion control and soil amelioration.

From having been a minor practice in the 1960's and early 1970's, planting of multi-purpose tree species exceeded 2.6 million hectares during the 1980's, thus reaching the same levels as the tree planting for industrial purposes (Lanly, 1992). These are conservative estimates which only give an indication of the extent of multi-purpose tree planting. Over 2000 tree species have been put to non-industrial use (Simmons, 1992).

Non-industrial tree species have consequently received increased attention from national and international organisations, especially those dealing with rural afforestation programmes in developing countries (Venkatesh, 1988). In many of these countries, for example, the emphasis on donor assistance and national development tree planting programmes has changed from industrial plantation forestry to community forestry programmes, involving tree planting in village woodlots, wastelands, shelter-belts and agroforestry on farmlands. (World Bank/FAO, 1981). In Africa, FAO has also established, with the French Funds Trust, a scheme in 12 countries for conservation, seed collection and evaluation and improvement of genetic resources of multi-purpose trees (Venkatesh, 1988).

The rapid development of agro-forestry and community forestry programmes has emphasised the need for increased research on multipurpose trees, especially on aspects that can increase the overall productivity of the land use systems. A proven method for increasing forest productivity is the careful application of genetic knowledge (Bawa and Krugman, 1991). Tree breeding is therefore likely to play a major role in increasing productivity of these systems. Workshops on multi-purpose trees organised by IUFRO's Special Programme for Developing Countries in Asia, Africa, and Latin American countries identified genetics and tree improvement as a priority field in forestry research in these regions (Palmberg, 1986). Also, in setting priorities for research in Forestry in 1984, the World Bank identified forestry in relation to agriculture and rural development and forestry in relation to energy production and use as the major priority areas for research (Keith and Carson, 1984). In both areas, specific priorities identified were choice of tree species, seed supply and breeding. This emphasis on tree improvement has led to initiation of breeding programmes for a number of multi-purpose tree species.

1.2 The study species

Grevillea robusta A. Cunn. ex. R. Br., commonly known as the Silky oak or the Southern Silky oak is one of the most important and successful multipurpose tree species used for agroforestry and community forestry programmes in the tropical highlands of East and Central Africa. The species is successful for several reasons: it is very easy to grow and interferes little with agricultural crops when grown in combination, it provides shade without significant effects on other crops and provides a wide range of products and services. The species is popular as an ornamental because of its attractive features such as fern-like leaves, brightly coloured flowers and racemose branching system (Owino, 1992).

Although *G. robusta* has been successful in many countries to which it has been introduced, the origins of almost all overseas plantings of the species are virtually unknown. It is probable that most of these introductions are likely to have been from a few trees, and therefore of very narrow genetic base (Harwood, 1992). The limited range of seed sources used and the narrow genetic base of the species introduced in these countries means that it is unlikely that the full potential of the species has been realised.

Planting programmes established from a small number of individuals will represent only a small spectrum of variation of a species throughout its natural range (Stern and Roche, 1974). Breeding programmes based solely on such sources will reduce variation even further. Thus, there is need to identify the full genetic potential of each species used. Quantification of natural genetic variability within the species must be the first step in developing any intensive breeding programme.

It is important to note that genetic development of a species should not be confined to enhancing productivity only under favourable environmental conditions. Many countries, particularly the densely populated, less developed ones are being forced to turn towards arid and semi-arid lands for agricultural expansion and new settlements. *Grevillea robusta* is being increasingly planted in arid and semi-arid zones of many tropical countries (Goor and Barney, 1976). The use of this species will therefore not only be important in high potential areas, but also in these arid and semi-arid areas where water is the limiting factor for growth of trees.

Genetic improvement of characteristics that may confer resistance to water stress in *Grevillea robusta* will greatly improve establishment and growth of the species in the arid and semi-arid zones. Several characteristics that may be associated with resistance to water stress in the species include morphological, such as deep rooting systems, pubescence and other leaf traits and, physiological characteristics such as leaf shedding and adjustment in tissue water relations. Given the wide climatic variation over the natural range of the species, genetic variation might be expected in characteristics such as growth rate and resistance to drought. No studies have been conducted to date on the extent of genetic variation within the species in such attributes (Harwood, 1989a).

1.3 Aims and scope of the study

The studies described in this thesis have assessed variation in growth and productivity in provenances of *Grevillea robusta*. Eleven seed sources from within the natural occurrence of the species were used to test the existence of variation between and within provenances in seed and seedling growth traits.

Four of these provenances were selected for a later study of variation in growth and productivity under water stress conditions. An additional provenance not used in the first study was also included in this study. The provenances selected were from two contrasting habitat types, the riverine and the dry upland, which differ in moisture availability.

The aims of the experiments can be summarised as: to determine the patterns of variation in growth characteristics between and within provenances of *Grevillea robusta* grown from seed collected at different altitudes and in different habitat types within the natural range of the species and to test the importance of geographic location and climatic factors of seed source on growth of seedlings. Specific objectives are:

To test the provenances of *Grevillea robusta* for variation in seed and seedling growth characteristics, early growth and dry matter production.

To examine the partitioning of genetic variation between and within the provenances in growth characteristics and to estimate their heritability.

To test the populations of *G. robusta* for presence of clinal and ecotypic variation and to examine correlations among seed and seedling growth characteristics.

To determine the morphological response of *G. robusta* seedlings to water stress and to evaluate variation in this response between and within populations of the species.

1.4 The layout of the thesis

This thesis is divided into two sections: The first one deals with variation in seed and seedling growth characteristics between and within the eleven provenances of *Grevillea robusta.* This section is composed of Chapters 2 to 7.

Chapter 2 outlines the main characteristics of the species, including its distribution, ecology and growth and uses. The importance of the species as a multipurpose tree is discussed.

A review of the importance of genetic variation in tree breeding and factors determining this variation with special reference to *G. robusta* are discussed in Chapter 3.

Chapter 4 presents details of materials, experimental methods and procedures and statistical methods used in the analysis of data.

The results and discussions of the study of variation in seed and seedling characteristics in *G. robusta* are presented in Chapter 5. These include the partitioning of variation between and within provenances for the different traits assessed.

Estimates of heritability of different traits and phenotypic correlations among seed and seedling growth traits and their significance in *G. robusta* are presented in Chapter 6.

Section one ends with a presentation in Chapter 7 of general discussions and conclusions to the study of variation in *G. robusta.*

Section two deals with the study of variation in growth characteristics in response to water stress between the five provenances from the two contrasting habitat types of the natural occurrence of *G. robusta.* Chapter 8 introduces the study, whereas Chapter 9 presents the results of the experiment. Chapter 10 presents a general discussion of the results.

Chapter 11 presents the overall discussions and conclusions resulting from both studies.

SECTION I

CHAPTER 2

THE SILKY OAK:- THE TREE, ITS NATURAL DISTRIBUTION, ECOLOGY, GROWTH AND USES

2.1 Botanical description and taxonomy

Silky oak (*Grevillea robusta* A. Cunn. ex R. Br.; synonym - *Grevillea umbratica* A. Cunn.) is a tall tree, which grows to 40 m in height and 100 cm diameter. It has light green fern-like leaves, pinnately lobed with secondary or tertiary lobes. The name Silky oak is derived from the silky hairs on the lower surfaces of leaves. The wood resembles European oak, hence the name.

The species is in the genus *Grevillea,* sub-family *Grevilleoideae* of the *Proteaceae* family. It is taxonomically isolated in the genus and lacks close relatives. However, it appears to have some connection with *Grevillea exul* Lindl. (McGillivray, 1993). The genus forms a major group of the *Proteaceae* family, with over 260 recognised species, of which *Grevillea robusta* attains the biggest tree form. The species is semi deciduous and leaf fall occurs during dry winter and spring seasons (July to October). Morphological characteristics of the species have been described in detail elsewhere (eg., Harwood, 1992; McGillivray, 1993).

2.2 Natural distribution

Grevillea robusta is native to the Australian sub-tropical and tropical forests of coastal New South Wales and Queensland (see Figure 2.1). Its recorded latitudinal limits span 470 km, from 24° 30' to 30° 10' south, and it is distributed over a wide range of conditions. The altitudes range from near sea level to 1120 metres, about 160 kilometres from the coast.

The region receives a mean annual rainfall ranging from 720 to 1710 mm, with the heaviest falls taking place during the late summer months of January to March. The mean rainfall of the driest month ranges from 30 to 57 mm. The east-west decrease in rainfall is sharp, especially on coastal ranges which produce localised areas of higher rainfall and rain-shadows. Topography modifies the effectiveness of rainfall. On higher altitudes, the effectiveness of rainfall is enhanced by lower temperatures and increased clouds and mist (Baur, 1957). The mean maximum temperature of the hottest month is about 28 to 30 °C, and the mean minimum of the coldest ranges from 2 to 8 °C.

Grevillea robusta is found in a wide range of conditions. Its major occurrence is in two habitat types (Harwood, 1992); first, on soils with high fertility and good moisture availability, in the Castanospermum-Waterhousia-Floribunda alliance (Floyd, 1979). These are a group of communities forming narrow fringes along river banks and streams and variously known as gallery or riparian rainforests. Here, the species occurs in association with Black Bean (*Castanospernum australe* A. Cunn.), Red Cedar (*Cedrela australis* L.) and Tulip Oak (*Tarrietia argyrodendron* Bailey) (Swain, 1928; Harwood 1992), and in association with *Podocarpus elatus* and *Melia azedarach* .

Secondly, the species is found on less favourable localities along rivers or in the drier creeks and dry exposed sites, in association with River she-oak (*Casuarina cunninghamiana*) and in the more mesic Araucarian vine forests dominated by *Araucaria cunninghamii*. In these areas, the species develops as small pure groups in jungle clearings and pockets or under eucalypt formations along banks and beds of streams (Swain, 1928).

It is also found as a single occasional tree of the hoop-pine-Yellow wood-Ash associations, with a preference for deep, soft red basaltic or black alluvial soils (Swain, 1928). These forests, usually remote from rainforest stands are found away from rivers, on basalt derived soils of medium to high fertility, where the species occurs with or without *Casuarina cunninghamiana*.

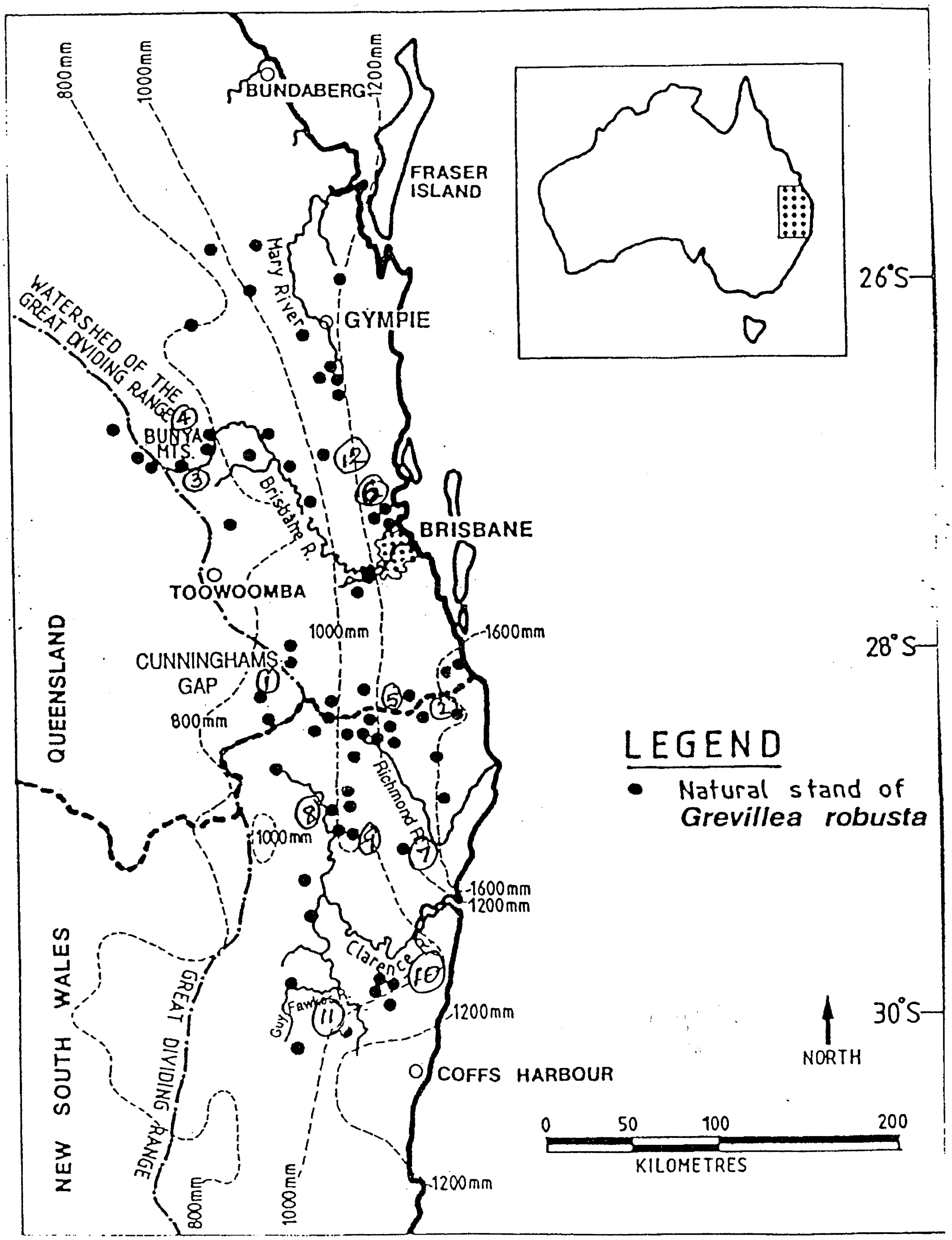

Fig. 2.1. The natural distribition of *Grevillea robusta* in Australias East coast. Provenances used in the studies are shown.

Source: Harwood, (1992)

Provenance identities[1]:

1. Emu vale, 2. Nimbin, 3. Bunya mountains, 4. Porters gap, 5. Albert river, 6. Wivenhoe, 7, Rappville, 8. Duck creek, 9. Mummulgum, 10 Mcphersons, 11. Boyd river, 12. Conondale.

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[1] These identities do not correspond to those used in other parts ofthe thesis and are used for identification purposes only.

These forest communities are floristically complex and related to the tropical rainforests in the Indo-malaysian region. The major factors associated with the distribution of rainforests in the areas of natural distribution of *Grevillea robusta* are geology, soils, topography and fire (Baur, 1957). There are two major types of geological formations within the species' natural range; first, ancient coal measures of Gympie formations, (ie., sandstones, shales) and secondly, recent Tertiary intrusions. When growing on the recent intrusions, the species shows a definite preference for red loam soils (Webb and Tracey, 1981). These are mostly found on banks above alluvial levels or as tableland undulating plains and slopes.

Forests within the natural occurrence of *Grevillea robusta* have been considerably reduced by exploitation for timber and agricultural development and by fire. By the time of European settlement in Australia about two hundred years ago, rainforests in the region were estimated to cover about eight million hectares, but only two million hectares remain today. Extensive logging of the species has been carried out such that it no longer exists in commercial quantities (Webb and Tracey, 1981).

2.3 Ecology

In its natural habitat, *Grevillea robusta* colonises rainforest margins and disturbed sites such as logging tracks, old cultivations and levee banks of streams. The species regenerates singly, or in small patches of uniform age and persists only as scattered emergents in advanced stages of rainforest succession (Webb *et al.*, 1967). In Queensland, the species regenerates freely into adjacent plantations of *Araucaria cunninghamii* and along edges of the rainforest. Natural regeneration usually follows fire, tree fall, or other disturbance that provides an opening in the forest canopy (Harwood, 1992). When planted under favourable conditions outside its natural range, the species can be an aggressive coloniser and is considered a weed in some parts of the world eg., in Hawaii (Nelson., 1987).

Fire appears to have restricted the natural range of *G. robusta* (Harwood, 1992). The species can survive small ground fires but is killed outright by hot fires that occur in dry conditions. It regenerates vigorously from the root collar after limited fire damage (Swain, 1928).

2.3.1 Optimum growth conditions

Within its natural range, *Grevillea robusta* grows best in warm temperate to sub-tropical regions with a mean annual temperature of about 20 °C and a mean annual rainfall of 700 to 1500 mm. Mature trees can withstand occasional frost, but young plants are frost sensitive. The species grows well in many types of soil but is sensitive to root checking (Swain, 1928) and does not perform well on heavy clay soils or in waterlogged conditions (Harwood and Booth, 1992).

Outside its natural range, *G. robusta* can tolerate heavier frosts and drier conditions than occur in its natural habitat. In Australia, for example, the species has been planted from temperate Tasmania to tropical north Queensland, including Canberra, where temperatures fall as low as minus 7 °C, and in drier semi arid localities such as Alice Springs in the Northern Territory, where mean annual rainfall is as low as 380 to 500 mm (Webb *et al.*, 1967).

2.4 *Grevillea robusta* as an exotic

Grevillea robusta has been planted outside its natural range for over 160 years. It was first introduced to Ceylon in 1830 and since then to more than thirty other countries around the world; in Africa, Asia, Central America, Europe and India. The species has been reported as growing successfully in a wide range of climatic and environmental conditions; in high rainfall to low rainfall semi arid tropical climates, mediterranean and temperate climates. The species performs well in sub-humid to humid tropical lands of Africa, Asia and Central America. Interestingly, of the Australian species of the family *Proteaceae* whose ranges extend from the rainforests in the humid tropics to open arid environments, only *Grevillea robusta* has been successfully planted as an exotic.

The species was first cultivated as an ornamental and was later found useful as shade in tea and coffee plantations at altitudes between 1200 to 2300 m eg., in Ceylon and East Africa. (The shade giving characteristic of the species has also been observed in its natural environment, where it is a natural shade giver to *Flindersia oxyleyana* (Anon., 1967). Its use as coffee and tea shade has declined as research in several African countries has shown that shading usually lowers tea and coffee yields and sometimes results in outbreak of pathogens (Arap Sang, 1987). Despite this, the species is being increasingly used as an agroforestry tree component, for on-farm planting and other general purpose planting in Brazil, East Africa, and India.

Studies in several countries suggest that growth and performance of *G. robusta* varies a great deal and may depend on a combination of site factors. It reaches its best development on middle altitude areas with high rainfall and deep, fertile soils. Its rate of growth in the tropics is lower at higher altitudes. For example, in Kenya (Owino, 1992) and in Tanzania (Shehagilo, 1992), growth rate of the species often declines with increasing altitude of the planting site, given soils of similar fertility. Similarly in Ethiopia, the species performs poorly at altitudes above 2000 m, but grows vigorously below 2000 m (Forest Research Centre, 1986, cited in Okorio and Peden, 1992). Lower temperatures at higher altitudes may be associated with low rates of growth.

As mentioned earlier, the species performs well on deep soils and its growth is severely restricted by shallow soils, clay soils and waterlogged conditions. This is also observed when the species is planted outside it natural range. Problems with boron deficiency and manganese toxicity on very acidic soils (pH 4.2) have also been reported in several countries (Smith, 1960; Harwood and Amare, 1990). In Rwanda, die-bark problems affecting the species have been attributed to boron deficiency (Arap-Sang, 1987)

As in several other proteaceous genera, *Grevillea robusta* forms proteoid roots. These are unbranched, 2 to 2.5 centimetre long clusters of rootlets that occur at intervals on the main roots. The rootlets are not mycorrhizal, but are believed to be formed with assistance of soil bacteria, which produce substances that initiate their formation. They are thought to assist *G. robusta* and other members of the *Proteaceae* family to adapt to low moisture and low nutrient conditions (Wrigley and Fagg, 1989). Their large surface areas increases the efficiency of absorbing nutrients and soil moisture.

Growth performance of *G. robusta* is also sensitive to moisture availability. The species reaches its best development on areas with high rainfall (Kamweti, 1992; Thimma-Raju 1992). For example, Kamweti (1992) found higher mean volume production per tree, 1.8 m^3 at the age of 27 years (0.067 m^3 per tree per year) on a high rainfall site with a mean annual of 1245 mm. On an arid low rainfall site with 600 mm, the mean volume production per tree was much slower with 0.15 m^3 at the age of six years (0.025 m^3 per tree per year). In Uganda, the species maintained an annual height growth rate of 2.5 metres per year up to 20 years of age on favourable sites (Okorio and Peden, 1992).

Despite its performance being related to moisture, the ability of *Grevillea robusta* to tolerate drought has been reported by several authors. The species has been described as ``extremely drought hardy, defying insolation and regenerating in open sun, flourishing in the hottest sites in the most arid to the most humid air of its climatic range" (Swain, 1928). In the early years of its cultivation, the species was also observed to be hardy, being used as an ornamental in the most arid towns of Australia. The species has also been observed to tolerate drought in Peru, where it grows in areas with rainfall as low as 600 - 700 mm per annum. In many tropical countries, the species has grown satisfactorily in areas with rainfall of 400 - 600 mm per annum (Harwood and Amare, 1990).

2.5 Importance and uses of *Grevillea robusta*

In its native Australia, *G. robusta* is no longer of economic importance. Logging of the species has stopped and without plantations, it is unlikely to become important. Past uses in Australia include making of cheese and wine casks and for timber and furniture.

Outside Australia, *G. robusta* has proved a successful tree species component in agroforestry systems in many parts of Africa (Harwood, 1992; Kalinganire and Zuercher, 1992) and is a top priority tree species for agroforestry development in the humid highlands in Eastern and Southern Africa (Owino 1992). In a survey of agroforestry tree species carried out in 1989, the species was found to be the most preferred species by farmers out of the twenty one short-listed (Ongugo, 1992).

The increasing interest in *G. robusta* in many countries within the tropics has resulted from rapid agroforestry and social forestry developments in the region. Moreover, the species is easy to grow and tend, can withstand heavy pollarding and pruning and exhibits little negative competitive effects with agricultural crops when grown in association with them. In Burundi, for example, no significant reduction was found in banana yield after thirty months of inter-cropping with *G. robusta* trees (Warner, 1993). Also, a trial in Rwanda showed that the maximum crown cover of *G. robusta* was only 20 % when the species was grown at an initial stocking of 400 to 600 trees and reduced at age nine years to 250 to 300 trees per hectare (Kerkhof, 1992). This interfered little with growth of agricultural crops. Moreover, when the species is grown in an intercrop mixture, the roots penetrate deeply into soil layers far below the zone utilised by agricultural crops. The roots also recycle nutrients from these levels back into the soil surface.

Grevillea robusta is also planted for other reasons. For example, it is used as an ornamental tree in many cities in Australia and around the world and also as fuelwood. Its wood has a moderately good calorific value of 4.90 Kcal g^{-1} for the heartwood and 4.91 Kcal g^{-1} for the sapwood (Thimma-Raju, 1992) and produces good charcoal. The species also provides good yield of poles for general construction purposes and is also used for veneer and plywood production. The species is also planted as a honey yielder in the Mediterranean bee keeping zones (Goor and Barney, 1976).

The importance of the species in many rural communities is reflected, in part, by the demand for seeds. For example, in Kenya the demand far outstrips seeds supplied by the Kenya Forest Tree Seed Centre (Ongugo, 1992) and it is the highest of any tree species in Rwanda (Kalinganire, pers. comm[1] 1993). A similar situation has been observed in Tanzania (Shehagilo, 1992).

To summarise, within the range of environments suitable for *G. robusta,* intensive on-farm use of the species can make densely populated rural areas self sufficient in wood products with minimal interference with food crops. This integration of wood production into farming systems avoids land-use conflict and distribution problems involved in setting aside areas for plantation forestry to meet rural wood requirements (Harwood, 1992).

2.6 Wood properties and utilisation

The wood of *Grevillea robusta* is of good average quality and moderately durable. Mature wood has a basic density ranging from 510 to 640 kg m^{-3} and specific gravity of 0.54 to 0.66 (Anon., 1980). The species produces yellowish brown heartwood with a characteristic oak-like grain Boland *et. al.,* 1978) The wood contains a very high proportion of large prominent ray parenchyma cells that contain high amounts of solutes (Samidi, 1993).

The heartwood is used for high quality furniture, light construction and joinery. It saws and machines with ease but ray tissue tends to lift with small

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[1] Forest Research Officer, Institute des Sciences Agronomiques du Rwanda. Butare, Rwanda.

moisture change (Samidi, 1993). It is also used for veneer and plywood production and for fuelwood. In Brazil, the wood is used for packaging.

Studies in India have shown that *Grevillea robusta* may have the potential for production of dissolving pulp (alpha-cellulose) (Madan and Tandon, 1991). Dissolving pulp is a highly purified form of cellulose used for manufacture of rayon fibre, a clothing material. The strength properties of viscose rayon fibre prepared from the species' dissolving pulp are comparable to commercial rayon fibre made from other sources .

2.7 Propagation

Grevillea robusta is easily propagated from seeds, although the seeds do have low viability. The species bears fruit at a very early age, six to eight years, with the heaviest seed crop produced between the ages of 20 to 40 years. It flowers in October and begins to shed seeds from the follicles by December of the same year.

Pre-treatment is not essential for seed germination but Okorio (1989), cited in Bunyinza, (1992) found that soaking seeds in water for 24 hours did improve germination. Under normal storage conditions, seeds remain viable for only a few months, but dried and refrigerated, they have been reported as remaining viable for up to two years (Wanyondu, 1992).

The seeds are commonly germinated in fertile loam soils with a shallow covering of sand and pricked into polythene tubes on appearance of first true leaves. Seedlings are ready for planting out when they are about 20 to 40 cm tall, about four to eight months after germination. The species can be propagated vegetatively.

Although G. *robusta* regenerates naturally, the seedlings fail to establish within the root zone of the parent tree (Moore and Keraitis, 1966). The roots are thought to produce auto-allelopathic chemicals or other water-transferable factors that inhibit natural regeneration of its own kind. (Webb *et al.*, 1967). In the affected areas, the seeds germinate well but the seedlings become stunted, chlorotic and exhibit a characteristic blackening of leaf tips before perishing within a few months after germination. However, the species does not inhibit regeneration or growth of other tree species growing under it, either in plantations or in its natural environment.

2.8 Growth rate

Grevillea robusta is characterised by an initially fast growth rate which decreases as the tree matures. It generally grows to a height of 30 to 40 m but is a somewhat short lived species and growth almost stops at the age of 50 years.

When grown in plantations, the rate of growth is extremely rapid, reaching 1.5 to 3 m within the first year and about 10 m during the first six years and where climate and soils are suitable, rates of up to 2 m in height and 2 cm diameter per year for the first five years have been reported (Harwood and Amare, 1990). A mean annual increment of 14 m^3 was achieved over 43 years in Hawaii and a mean annual increment of 10 m^3 per hectare is commonly achieved over 10 to 20-year rotations at stocking densities of 500 to 1200 stems per hectare (Harwood and Amare, 1990). Rates of growth are slower under less favourable conditions. Owino (1992) claimed that the species is not suitable for large scale plantation development and is predominantly an isolation ideotype but there is evidence that under optimum spacing, the species has grown as well as other plantation tree species in Rwanda (Kalinganire and Hall 1993) and in Hawaii (Burns and Menandra, 1988).

2.9 Pests and diseases

On wetter and hotter extremes of its climatic range, *Grevillea robusta* is prone to fungal diseases and insect attacks (Harwood and Booth, 1992). The fungal pathogens include *Corticium salmonicolor*, *Phyllostica*, *Cercospora*, *Diplodia*, and *Dothiorella* spp. It is also liable to attack by a root fungus disease, *Phytophthora cinnamomi*. *Asterolecanium pustulans* is a serious scale insect pest in the Caribbean region. Termite attack is also a serious problem in drier parts of Africa. It's wood is liable to attack by *Ambrosia* and *Lyctus* beetle attack.

2.10 Genetics and tree improvement

As noted earlier, the importance of non-industrial or multi-purpose trees to many communities in rural developing countries has led to initiation of breeding programmes for some of these species (Simmons, 1992). Tree improvement programmes for *Grevillea robusta* have been initiated because of increased awareness of the potential of the species in rural afforestation in some developing countries and especially the extent to which the species is preferred by farmers. Among the characteristics suggested for improvement are growth rate, tolerance to various stresses such as drought, heat and termite attack because, as pointed out in Section 1.2, the species is being increasingly planted in arid and semi arid areas.

Harwood and Owino (1992) have suggested two possible ideotypes to which genetic improvement programmes might aim. Isolation ideotypes (broad crowned) are likely to be suitable for shade, while crop ideotypes (narrow crowned) are likely to be suitable for row and boundary planting and possibly woodlots. The authors have suggested a detailed strategy to be followed in genetic improvement of *G. robusta*. This includes establishment of provenance and progeny trials in locations representative of major climatic zones in which the species is important, followed by a heavy selective thinning to remove inferior phenotypes. The remaining stand should serve as a seed production areas. This strategy would allow local production of genetically improved seed within eight to ten years (Harwood and Owino, 1992).

CHAPTER 3

IMPORTANCE IN FOREST TREE IMPROVEMENT AND FACTORS DETERMINING DISTRIBUTION OF GENETIC VARIATION WITH SPECIAL REFERENCE TO *GREVILLEA ROBUSTA*: A LITERATURE REVIEW

3.1 Introduction

Natural variability existing within a tree species, especially in its natural populations constitutes the basis for selective improvement. Most forest trees contain great variability in economically important traits as growth, stem straightness, specific wood gravity and adaptability to stress such as drought.

Consequently, tree breeding has been practised in a number of species, particularly pines, eucalypts, spruces and poplars (Burley *et al.*, 1986). The stages of tree breeding have been described by a number of authors (eg., Namkoong *et al.*, 1980; Zobel and Talbert, 1984). The first step is the identification of the most suitable species and their provenances followed by definition of the selection criteria.

The selection criteria and the intensity and extent of selection are in turn dependent upon the population genetic structure, which is described as the pattern or partitioning of genetic variability between and within populations of a species (Bawa, 1976). The term population genetic structure has been also described as the sum total of the ecological and genetic relationships among individuals of a species and the populations they comprise (Jain, 1975). Success in improvement of such species depends on thorough knowledge of population structure and inter-population variation (Bawa, 1976). A breeding strategy inappropriate to the actual population genetic structure will reduce the genetic gain (Gurries and Ledig, 1989).

Thus, the understanding of the organisation of genetic variation within a species is of critical importance for devising an optimum sampling strategy for breeding purposes. Since selection involves sampling, genetically similar populations can be sampled less extensively while highly variable species must be sampled more intensively. Of the many selection methods and sampling options available to the breeder, the most appropriate will depend upon the type of genetic variation existing within a species, especially in its natural populations (Zobel and Talbert, 1984).

The genetic structure in *Grevillea robusta* is poorly understood but Harwood, Bell and Morans' (1992) study of the isozymes of 19 provenances of the species revealed the existence of substantial genetic variation in its natural occurrences. However, patterns of variation in morphological characteristics are poorly understood and few studies have been made of the extent of variation in physiological traits such as growth rate and drought and frost resistance either in the species' natural populations or in the land races (Harwood, 1989a).

In a provenance trial of five natural provenances and several land races of *Grevillea robusta* in Rwanda, Kalinganire and Hall (1993) found significant variation in height and diameter growth and in branch growth and crown depth. The natural provenances grew faster than the land races, suggesting wider genetic variation in the natural provenances than in the local land races. Kalinganire and Zuercher (1992) have also reported interim results of another provenance trial of the species in Rwanda. They found significant differences in growth between provenances from the northern part of the natural range of the species.

It seems clear that where *G. robusta* has been introduced and naturalised, the obscure and possibly the restricted origin of the founder population makes the possibility of `founder effect' real (Harwood, 1992). The term `founder effect', coined by Mayr (1963), refers to the establishment of a new population from a few original founders, which carry only some of the total genetic variation of the parent population. In a review of founding events, Clegs and Brown (1983) concluded that most such events are associated with a decline in total variation relative to that of the parent population.

The reduction of genetic variation due to problems associated with founder effects has been documented in several plant species. For example, studies of *Emex spinosa* which was introduced in Australia in the mid 1930's from the Mediterranean basin indicate a loss of genetic variation that is associated with colonisation (Marshal and Weiss, 1982). This loss was also associated with absence of migration as a source of genetic enrichment and with selection pressures in the new environment. For *Leucena leucocephala,* the most planted populations in many countries were derived from one or a few cultivated trees in North Mexico, resulting in most areas of the tropics being planted with an extremely narrow based germplasm of the species (Hughes, 1989). Similarly, narrow genetically based introductions of *Acacia mangium* occurred in Malaysia, resulting in a progressive decline in the performance of trees in successive generations (Sim, 1989) and, a land race of *Gliciridia sepium* developed in Sri-lanka based on progeny of a single tree performed poorly compared to the wild population in a provenance trial of the species. Similar problems have also been documented in *Acacia tortilis* in India.

A study of genetic variation in several natural provenances and African land races of *Grevillea robusta* showed that after several generations, the African land races of the species had lower levels of mean heterozygosity and polymorphic loci than did natural populations. Mean expected heterozygosity of the land races was 0.079, 26% lower than that of the natural populations, 0.107 (Harwood *et al.*, 1992). The authors suspected the land races to have originated from small founder populations, resulting in lower genetic variation. This is consistent with the founder effect noted earlier.

The restricted genetic variation in founder populations does not appear to reduce population viability or colonisation success. However, attempts to genetically improve a species incorporating only the land race populations may not realise any substantial gains in a breeding programme because of the reduced genetic variation. Under such circumstances, importation of other seed sources may be necessary to broaden the genetic base of the species.

3.2 Factors determining patterns and distribution of genetic variation

For effective use and management of genetic resources, it is important to understand not only the distribution of genetic variation within a species, but also the environmental and species characteristics that influence this distribution. Factors determining the structure and extent of genetic variation in plant species have been identified as the environment, the geographical range of the species, population size, primary mode of reproduction, the breeding system, seed dispersal mechanism and the community type in which the species occurs. These factors may not be mutually exclusive but may combine to determine the structure of genetic variation (Bawa and Krugman, 1991).

3.2.1 Geographic range and environmental heterogeneity

3.2.1.1 The effect of geographic range of a species

The geographic range and environment of a plant species have a major influence on the amount of genetic variability in the species (Loveless and Hamrick, 1984). Plant species with large geographic ranges often develop locally adapted sources that are genetically distinct. These sources exhibit morphological and physiological characteristics that make the populations suitable for growth under the local climatic conditions. Studies of genetic variation in most forest tree species have found differences to be greatest in species covering wide geographical areas (eg., Wright, 1976). Nevertheless, substantial variation also can occur in species having small geographic ranges. Strong selection forces in a species' environment can create patterns of genetic differentiation on small local scales (eg., Bradshaw, 1984; Liu and Godt, 1983). This is because the pattern of differentiation depends on the outcome of the interplay between natural selection (tending to enhance the difference) and migration (tending to reduce the difference). However, due to the sedentary nature of plants, migration can easily be overlaid by selection. As a result, patterns of differentiation in populations tend to follow environmental patterns very closely (Bradshaw, 1972).

The existence of isolated populations within a species' range may increase differentiation within species as gene-flow will become small or non existent. It has also been postulated that marginal populations in ecologically peripheral habitats of a species' range may be subject to strong directional selection pressures (Grant, 1981; Stern and Roche, 1974), resulting in these populations being generally differentiated from the rest of the population.

3.2.1.2 The effects of environmental heterogeneity

The evidence of the role of heterogeneous environments in producing genetic differentiation by disruptive selection is extensive (see Antovonics, 1971). Significant genetic heterogeneity has been shown to exist among geographically distinct populations, among subdivisions of local populations or progeny of individual plants. Individual plants may respond to different environments in terms of flexibility in size, morphology and physiological behaviour (Bawa, 1976).

When trees of different origins are grown in common gardens, they show marked differences and patterns in growth traits that may reflect the pattern of source the environment (Ennos, 1983). These patterns of genetic differentiation are normally viewed as either continuous (clinal) or discontinuous (ecotypic). In cases where variation in traits linearly follows environmental gradients, the relationship is clinal and where variation in traits is related to changes in the environment but does not follow linear gradients, the relationship is ecotypic. These patterns are not qualitatively different and may result from adaptive strategies of the plant population which, in turn depend upon niche distribution and differences in selection pressures within the species' habitat (Hedrick *et al.*, 1976).

3.2.1.2.1 Clinal variation

The most acceptable idea of variation in forest trees is that climate has a continuous variation pattern and tree growth is related to climate. Plant species tend to exhibit gradients in response to broad climatic factors eg., day-length, temperature and rainfall because they must match their growth periods with the length of the growing season, as well as extreme conditions such as cold, drought and frost (Burley, 1984; Peng *et al.*, 1992). Consequently, many forest trees exhibit marked variation between individual populations across altitudinal, latitudinal, and longitudinal gradients especially for traits related to adaptation such as drought hardiness and phenology. In some species, ecotypic variation may not be related to any identifiable environmental factors and is presumed to be due to stochastic factors (Hedrick *et al.*, 1976).

3.2.1.2.2 Ecotypic Variation

The term 'Ecotype' was originally proposed by Turreson (1922a, as cited in Quinn, 1987) as `the ecological unit to cover the product arising because of the genotypical response of an eco-species to a habitat'. Plants with large geographical ranges often develop locally adapted populations that are genetically distinct. These ecotypes exhibit morphological and/or physiological characteristics that make the local populations suited for growth under the local climatic conditions they experience. Ecotypes develop in response to distinct environmental factors. Ecotypic variation requires the existence of genetic variation, differing selective pressures in various portions of the species range, and isolation by distance (Bradshaw, 1984)

Ecotypes presumably represent discontinuous genetic variation, correlated with specific habitats. However, according to Quinn (1987), the term ecotype lacks consistent use today and several other authors (Langlet, 1971; Quinn, 1978) have marshalled further arguments against its use. They have argued that ecotypes are not ecological or evolutionary units and that each population is a unique realisation of the genotype-environment interaction.

3.2.2 The role of the successional stage of a species

The structure of genetic variation has also been discussed in relation to the successional stage in which the species occurs (Mardsen and Blake, 1977; Baazaz and Carlson, 1982). These authors have argued that variability in environmental factors of early successional species such as light, temperature, moisture and nutrients are usually greater than that of late successional species and this should be reflected in greater variation in the former group. As an early successional species, some populations of *G. robusta* must face highly variable environments and it is therefore reasonable to expect them to contain large amounts of variation.

3.2.3 The role of breeding system and mode of pollination

Plants display a variety of breeding systems that differ in their influence on the mating patterns and population genetic structure (Richards, 1986). The mating parameter with the largest influence on genetic structure is the selfing rate. Breeding systems in turn are commonly determined by the pollination mechanisms.

The role of breeding systems in population differentiation has been described in detail. Electrophoretic data compiled over 20 years suggest that high levels of genetic variation exist in populations of out-crossing plants (40 - 70 % polymorphic loci) (Hamrick *et al.*, 1979). These plants maintain higher levels of intra-population variation than inbreeding species which show low levels of variation within populations, but high levels between populations.

The estimates of breeding systems of two natural provenances of *Grevillea robusta* were 0.96 and 0.86 (Harwood *et al.*, 1992) suggesting that the species is largely out-crossing. The species is largely bird and mammal pollinated (Moran, 1992), a characteristic consistent with high out-crossing rates. Despite these high rates, the potential for inbreeding is not negligible as low levels of self fertilisation do occur in some trees. This contrasts with some land race populations in Kenya, which have been found to have a predominantly self fertilising habit (Owuor and Oduor, 1992).

The relative amounts of out-crossing in a species have also been found to be correlated with the density of its populations. Dense populations have lower out-crossing rates than less dense populations, presumably due to presence of intra-family inbreeding in the dense populations (Ellstrand *et al.*, 1978). Again, it has been observed that *G. robusta* occurs in disjunct populations, and this confirms the likelihood of high levels of outcrossing.

3.3 Partitioning of variation between and within plant populations

The total genetic variation maintained within a species can be partitioned hierarchically, according to the way it is distributed among regions, populations and individuals within populations (Chambers an Bayless, 1883). In most species with large continuous distributions, most genetic variation resides within populations and less than 10 % of the variation occurs between populations. (Hamrick and Godt, 1990). In *Grevillea robusta*, the proportion of variation attributable to between population differences was 15 %, as estimated by Harwood (1992).

3.4 Methods of estimating genetic variation

Genetic variation can be detected by looking at discrete heritable differences between individuals or by measuring continuous phenotypic variation for characters of interest and determining whether this variation has a significant heritable component. Several methods have been used to study genetic variation in forest trees. The most common methods include morphological, isozyme and more recently, biochemical methods and DNA restriction-fragment analysis. Each method may be used with different objectives, and the results obtained from each have different applications, theoretically and in practice. Although only one method will be used in the present study, others are reviewed briefly.

No particular method can unambiguously describe total genetic diversity of a species (Chambers and Bayless, 1983). For example, electrophoresis reveals only a small fraction of the protein variation of the genome (Boyle and Yeh, 1988) whereas morphological variation can be confounded by phenotypic plasticity (Bradshaw, 1965).

3.4.1 Morphological variation

The simplest assessment of genetic variation is by measuring morphological or phenotypic variation. This method is used to estimate genetic variation, based on the assumption that genetic material controls the expression of every plant characteristic. However, the actual phenotypic expression is the result of environmental modification. Most traits of economic importance in forest trees are quantitative, that is, they are determined by many interacting genes whose expression is modulated by the environment (Zobel and Talbert, 1984). Quantitative genetics interprets the phenotypic expression of any trait as the sum of genetic and environmental effects (P=G+E), where P is the phenotype; G and E is the genetic and environmental components respectively. By isolating and removing the environmental component of variation through adequate experimental design, it is possible to estimate the genetic component of variation in a trait of interest.

Measurement of morphology has an advantage of requiring neither breeding nor laboratory studies, and can be measured directly. Another advantage of studying morphological variation is that phenotypic characters may be ecologically adaptive because morphological differences are often accompanied by differences in important physiological characteristics that may influence adaptive characteristics. Studies of morphological characteristics in natural populations can therefore suggest potentially suitable habitats for planting.

Studies of genetic variation using morphological characteristics can be carried out by assembling and raising seedlings from different sources in a common environment, for example in a glasshouse, and assessing their growth. Through appropriate experimental design (eg., Matheson, 1990), variation can be partitioned into environmental and genetic components. Similar information can be obtained through provenance testing, by growing different seed sources and assessing variation in performance under field conditions, again using appropriate experimental design to isolate the environmental component of variation.

Measurement of morphological variation has in the past been supplemented by biochemical methods of studying genetic variation. Methods such as gas chromatography are used to assess differences in plant chemicals such as monoterpenes, for example Kinlock *et al.* (1986) has analysed variation in monoterpenes in *Pinus sylvetris.*

3.4.2 Isozyme variation

Gel electro-phoresis can be used to estimate the amount of polymorphism for loci coding for specific proteins. As a technique most frequently to study isozyme variation, it is reasonably cheap and can be applied to most plant species (Hamrick *et al.*, 1979) and is also free of environmental effects. Despite its usefulness and widespread application, there are some well-known limitations: For example, only genes of a single class, those encoding enzymes are analysed. Thus, electrophoresis reveals only a small fraction of the protein variation of the genome because less than 10 % of the DNA in eukaryotes comprises structural genes that produce proteins (Gotlieb, 1981). Isozyme diversity also may not be well correlated with other measures of genetic diversity such as morphological traits (eg., Moran *et al.*, 1989). For example, while quantitative characters often have geographic patterns suggesting adaptive significance, patterns of isozyme variability indicate that isozyme loci are not subject to the same selection pressures as morphological characteristics and thus may not show similar patterns. Morphological patterns tend to show greater levels of variation than do isozyme loci (Boyle and Yeh, 1988). Isozyme variation therefore provides little information on the patterns of distribution of adaptive characters and may not be useful for describing such patterns in plant species (Schwaegerle *et al.,* 1986). However, some studies (eg, Hamrick *et al.,* 1979) have shown that similar patterns can be detected for both isozymes and morphological traits.

Therefore, in the absence of species' performance data, decisions on the range of possible seed transfer or delimiting breeding zones would best be based on genetic study of morphological variation. On the other hand, measurement of the phenotype does not detect unexpressed genes and variation may be obscured by phenotypic plasticity of the individuals being examined (Bradshaw, 1972).

3.5 Genetic improvement in *Grevillea robusta*

Recognition of the importance of *Grevillea robusta* has led to the initiation of tree improvement programmes for the species. Harwood and Owino (1992) have set out an improvement strategy for the species (section 2.10). According to Kageyema (1978), development of an efficient improvement strategy requires genetic knowledge pertaining to the breeding population, its selection and sizes. The vast, yet unselected natural provenances of *G. robusta* provide wide possibilities for selection and improvement of the species.

Apart from tree improvement to produce the maximum amount of tree products and services, improvement of agroforestry species also should consider farmer's special needs and services, compatibility with agricultural crops and sustainability of land productive capacity. Harwood and Owino (1992) have proposed a series of research steps for improvement of multi-purpose trees for specific agro-ecological and farm systems in East Africa. The steps involve among others, the identification of potentially useful species; identification of technologies with greatest potential for solving land use constraints; followed by listing and identification of multi-purpose trees for such technologies.

Such a programme was initiated in 1987, by the Multi-purpose Tree Improvement Programme for the highland zones of East and Central Africa. In 1988, network scientists established general species trials in four countries involving 14 species and provenances. Technology specific species trials were established for 10 species and from the assessment of these trials, four tree species were selected and improvement programmes established, among them *Grevillea robusta*.

3.6 Conclusions

This discussion of factors that influence the extent and distribution of genetic variation suggest that *Grevillea robusta* may possess wide genetic variation within its natural range and therefore the possibility of genetic improvement.

Previous studies have shown that overseas land races of the species may possess lower genetic variation than the natural populations and may suffer from associated founder effects. Because the economic importance of the species is mainly in its use as an exotic, evaluation of variation in the species may help identify the best means of improving the overseas land races, perhaps through selective enrichment of the genetic composition. This would require knowledge of variation and of the specific characteristics of the species and characteristics of its natural environment that determine the nature and extent of genetic variation.

CHAPTER 4

MATERIALS AND METHODS USED IN THE STUDY OF VARIATION IN SEED AND SEEDLING GROWTH CHARACTERISTICS IN *GREVILLEA ROBUSTA*

4.1 Introduction

Chapters 1 to 3 have identified the need for a study of variation in *Grevillea robusta.* This chapter describes the materials and methods used in the study. The traits assessed and the statistical analysis methods used are described.

4.2 Seed sources

Eleven provenances of *Grevillea robusta* were used in the initial study. The locations of the provenances used in the study are indicated diagrammatically in Figure 2.1. The seeds were obtained from the Australian Tree Seed Centre, Canberra, from collections made in 1989, 1990 and 1991 (Harwood, 1989b).

4.3 Growth conditions

The experiment was conducted at the Plant Culture Facility of the Australian National University in Canberra. The experiment was conducted under semi-controlled conditions in a glasshouse in which temperatures during the day ranged from 15 to 25 ^{o}C in winter and 18 to 30 ^{o}C in summer. The relative humidity ranged from 60 to 70 %. During winter (May to August), the natural day-length was extended to 16 hours using 400 watt/80mEs^{-1}m^{-2} Photosynthetically Active Radiation (PAR) metal halide lamps.

Seeds were sown in May 1992, in punnets containing a potting mix of equal proportions by volume of peat and vermiculite. The seeds were soaked in water for 24 hours prior to sowing. The punnets were set for germination on a heated bed under a regulated mist spray. Germination commenced after ten days and was complete by the seventeenth day after sowing. Seedlings were transplanted into eight centimetre diameter pots, (one seedling per pot) on appearance of the true leaves, and transferred to the experimental glasshouse. Five grams of Osmocote slow release fertiliser was applied to each pot.

Table 4.1. Provenances of *Grevillea robusta* used, their geographic locations and climatic details. (QLD - Queensland, NSW - New South Wales). Rainfall and Mean Annual Temperature were estimated using the BIOCLIM computer programme (Booth and Jovanovic, 1988).

Prov No.	Provenance	CSIR0 Number	Region	Altitude (m)	Longitude (E)	Latitude (S)	Rainfall (mm)	Temp (°C)
1	Emu vale	15873	QLD	545	152 17'	28 14'	825	16.9
2	Nimbin	17612	NSW	50	153 13'	28 38'	1505	19.7
3	Bunya mts	17633	QLD	1000	151 37'	26 54'	925	15.0
4	Porters gap.	17694	QLD	680	151 30'	26 45'	746	17.2
5	Albert river	17699	QLD	280	153 16'	28 16'	1438	18.0
6	Wivenhoe	17952	QLD	70	152 40'	27 19'	955	19.7
7	Rappville	17618	NSW	40	152 58'	29 07'	990	19.4
8	Duck creek	17614	NSW	200	152 33'	28 43'	1022	18.6
9	Mummulgum	17617	NSW	100	152 49'	28 50'	1095	19.2
10	McPhersons	17621	NSW	40	152 40'	29 48'	1054	18.9
11	Boyd river	17622	NSW	200	152 27'	29 53'	1020	18.4

4.4 Experimental design.

Seedlings were arranged in a Randomised Complete Block resolvable design with 8 replications, obtained using a computer generated layout. A resolvable design allows the data to be analysed either as Randomised Complete Block Design (RCB) or as an Incomplete Block Design (IBD). Single tree plots were used. Randomization was done at family level. Each provenance was represented by 3 families with 2 seedlings of each in each replication, giving a total of 528 seedling pots. The design allowed comparison of the two hierarchical levels of variation (among and within provenances).

4.5 Assessment and data analyses

4.5.1 Choice of traits

Grevillea robusta is planted as a component of agroforestry systems. As a multi-purpose tree, the species provides a wide range of products and services, such as fuelwood and timber and is also used for general purpose planting. Whatever the objective of planting, selection for improvement of the species should aim at traits that are associated with fast growth rates, survival and tolerance to various stresses. Such traits as height and diameter are important components of tree volume whereas leaves are the most important parts of the plant that are associated with growth. Leafiness in *G. robusta* is also important not only in determining the potential for photosynthesis, but also in providing mulch for prevention of run-off on slopes and also in providing off-season fodder. The size of the leaves, especially leaf area may therefore be used as an indicator of the potential of the plant for growth. Stem form is an important characteristic in multi-purpose tree species only when such species are grown mainly for timber.

The traits selected for assessment were those associated with growth and vigour. These are summarised in Table 4.2. Units of measurement and frequency of assessment are also given. In addition, seed characteristics also were measured.

4.5.2 Assessment

4.5.2.1 Seed characteristics

Seed weight, seed length and width were measured to give an indication of variation in seed size. For seed weight, a random sample of 25 seeds from four families in each provenance were measured to the nearest 0.05 mg, using a Mettler digital balance. These measurements were replicated four times. Provenance mean seed weight was calculated from seed weight of families comprising each provenance. Seed length and width were measured, to the nearest 0.5 mm, from a random sample of ten individual seeds per family, but due to insufficient samples in some families, only six provenances were assessed for this trait, compared to eleven for seed weight.

4.5.2.2 Height, mean internode length and diameter

The total height of each seedling was measured (in cm to the nearest mm) from the cotyledon level to the terminal bud. The cotyledon level was used as the datum to give a uniform base for assessment of height. Height growth measurements were carried out at two-week intervals. Four of these assessments made at 1, 3, 5, and 6 months of age were used for the analysis of variation in this trait. Mean internode length was derived by dividing the height of each seedling by the number of nodes.

Diameter was measured using a digital callipers (Digimat) at the point immediately below the cotyledon level. Two measurements were made at right angles to each other and averaged. These measurements were taken at four and six months of age.

4.5.2.3 Leaf characteristics

Leaf width has been used by others as a measure of leaf size but was not used in this study both because *Grevillea robusta* has compound leaves, and there is much variation both along the stem and along the same leaf.

4.5.2.3.1 Leaf area

A total of six fully expanded leaves were sampled from each seedling, two from each third of the stem length and their areas measured using a continuous belt automatic area-meter (Type AAM-5, Hayashi Denko Co. Tokyo). The leaves were then oven-dried separately at 75 °C for 72 hours and their dry weights obtained. The rest of the leaves from each seedling were also harvested and oven dried under the same conditions and their oven dry weights obtained. Total leaf area per seedling was calculated by extrapolation, Specific leaf area (mg/cm^2) was derived from basic leaf area and dry weight measurements. A total of three leaf area measurements were carried out at the age of two, four and six months.

4.5.2.3.2 Leaf length

The length of two terminal fully expanded leaves was measured (in mm, to the nearest 0.5 mm) in each seedling, from the tip of the leaf to the base of the petiole at two, four and six months of age.

Table 4.2. Traits assessed in glasshouse grown *Grevillea robusta* seedlings, their codes units of measurement and assessment interval. One seedling was harvested at each harvest.

Trait	Code	Units	Assessment at
Height 1 to Height 4	HT_1 to HT_4	cm	4, 8, 12, 20 and 24 weeks
Diameter 1 & 2	DM_1 to DM-2	mm	16 and 24 weeks
Leaf area 1 to 4	LA_1 to LA_4	cm^2	8, 16 and 24 weeks.
Leaf Length 1 to 4	LL_1 to LL_4	cm	8, 16 and 24 weeks.
Mean internode length	INTL	cm	24 weeks
Shoot dry weight	SH_WT	grams	8, 16 and 24 weeks
Root dry weight	RT_WT	grams	8, 16 and 24 weeks
Root:shoot ratio	R:S	-	8, 16 and 24 weeks
Stem dry Weight	STE_WT 1-4	grams	8, 16 and 24 weeks
Total biomass	BMASS	grams	8, 16 and 24 weeks
Specific leaf area	SLA	gcm^{-2}	8, 16 and 24 weeks

4.5.2.4 Harvesting and measurement of stem and root dry biomass

Three harvests were effected at the ages of two, four and six months. The following procedure was used for each harvest: The leaves were removed as described in the previous section and each seedling divided into component parts of stem and roots. Roots were separated from the stem at the cotyledonary scar and washed carefully. The two component parts were oven dried separately at 75 °C for 72 hours and their dry weights obtained. One seedling plot was harvested per family from four blocks for the first two harvests alternately and one seedling per family per block in the final harvest for all eight blocks.

4.5.3 Statistical analyses

4.5.3.1 Analyses of variance

Univariate analyses of variance were carried out separately for each trait to compare variation between provenances and between families within provenances, using GENSTAT 5 (Payne *et al.*, 1987) computer programme. The data were computed using single tree plot values. The error mean square was used for testing for the significance of the differences between provenances and between families within provenances. The statistical model, which specified families nested within provenances was:

$$Y_{ijk} = m + B_i + P_j + f_{(p)}k_{(j)} + e_{ijk}$$

Where Y_{ijk} = The observation on seedling k in block j from provenance i

m = The overall mean;

B_i = The effect of the ith block;

P_j = The effect of the jth provenance;

$f_{(p)}k_{(j)}$ = The effect of families within a provenance j; and

e_{ijk} = The residual (family x block effect).

Analysis of variance assumes independent, identically and normally distributed errors with mean zero and an unknown and fixed variance σ^2 (IIDN(0, σ^2)) (Box, *et al.*, 1978). Diagnostic checks for the assumptions of normality of the data and homogeneity of variance for each trait assessed were effected by analysis of residuals. Values that had a very large influence were omitted from the analysis and treated as missing values. Logarithmic transformations were used to normalise data that failed the diagnostic checks.

4.5.3.2 Estimation of variance components and intraclass correlations

Estimation of variance components was carried out to determine the percentage of the total variation in each trait attributable to the different sources of variation in the analysis of variance model. The variance components for each trait were estimated by equating the Expected Mean Squares of each source of variation to their corresponding calculated mean squares obtained from analysis of variance and solving for the components. Table 4.3 shows the model used in calculation of variance components. Provenances and families within provenances were considered as random factors to allow inferences of the results to be applied to a larger population of provenances and families in different provenances, whereas blocks were considered fixed and therefore did not contain any variance. For comparison, variance components were also estimated using REML (Restricted Maximum Likelikood) programme. Variance components were used for calculation of estimates of heritability of each trait.

The formulae used to calculate of proportions of total variance accounted for by each source of variation were: -

$$\frac{\sigma^2 p}{\sigma^2 p + \sigma^2 p(f) + \sigma^2 e}$$ For proportion of variance accounted for by provenances and

$$\frac{\sigma^2 p(f)}{\sigma^2 p + \sigma^2 p(f) + \sigma^2 e}$$ For proportion of total variance accounted for by families within provenances

where $\sigma^2 p$, $\sigma^2 p(f)$ and $\sigma^2 e$ are the estimated components of variance for provenances, families within provenances, and error, respectively. Intraclass correlations were calculated in the same procedure, but without the error component.

Table 4.3. Mean squares and their corresponding composition of Expected Mean Squares (EMS) r = number of replications (8) and f = number of families per provenance (3). Prov = Provenances

Source of variation	MS.	Expected Mean Squares
Blocks	MS_b	–
Provenances (Prov.)	MS_p	$\sigma^2 e + r\sigma^2_{p(f)} + rf\sigma^2_p$
Families within Prov.	$MS_{p(f)}$	$\sigma^2_e + r\sigma^2_{p(f)}$
Error	MS_e	σ^2_e

4.5.3.3 Multivariate analysis

The relationships between seed and seedling traits and the respective climatic and geographic parameters of the seed source were examined using simple regression analyses. Principal component analysis compared all traits assessed and was used to reduce into fewer components the dimension of variation in all traits combined.

4.5.3.3.1 Simple regression analysis

Correlation coefficients between means of all traits assessed in the study, with geographic and climatic parameters of seed source were calculated using GENSTAT's "correlate" directive (Payne *et al.*, 1987). Significance of the correlations was tested by comparing calculated correlation coefficient values with critical values obtained from the standard table at the 5 % level of probability.

4.5.3.3.2 Principal component analysis (PCA)

Principal component analysis (PCA) method has been used by several authors in similar experiments eg., Bleakey and Matheson (1992), Rehfeldt, (1993). In this study, the method was used to summarise variation in assessed variables by transforming correlated variates into new variates or components that were themselves uncorrelated and independent. The analysis took into account the correlation structure of the data. This transformation of one group of variates into a combined reduced set of variates reduces the dimension of variation between them, but retains the total variation in the original variates. Variates in each of the derived variables accounts for a proportion of the total variation which can be estimated (Sokal and Rolf, 1981). The derived variables are the principal components and the corresponding variances of the derived variables for each principal component are latent roots. All variables used in the analysis (measured or derived traits and geographic and climatic parameters of seed source) were considered equal and no distinction was made between dependent and independent variables (eg. Morgenstern, 1969).

4.4 Heritability estimates

Family and individual tree heritability values for each trait were estimated following the methods of Zobel and Talbert (1984), using components of variance calculated from the analysis of variance as shown in Table 4.3. The families within provenances were assumed to be half-sibs. The family variance component therefore estimated one-quarter of the additive genetic variance. Narrow sense heritability (h^2) was estimated as the ratio of the additive genetic variance to the total phenotypic variance for each trait (Falconer, 1989). The family heritability values of different traits were estimated using the following formula:

$$h^2 = \frac{4\sigma^2 f}{\sigma^2 f + \sigma^2 p + \sigma^2 e}$$

Where h^2 = heritability, $\sigma^2{}_f$ and $\sigma^2{}_p$ are trait family and provenance variance components respectivelly while $\sigma^2{}_e$ is the error component.

CHAPTER 5

PROVENANCE AND FAMILY VARIATION IN SEED AND SEEDLING GROWTH CHARACTERISTICS IN *GREVILLEA ROBUSTA*: RESULTS AND DISCUSSION

5.1 Introduction

This chapter presents the results and findings of the first experiment in the form of detailed analyses of assessments of individual traits, plus discussion of trends of variation in each. Results of simple regression analysis of individual traits on geographic locations and environmental factors of seed origin of the provenances are also presented with discussions of variation trends.

5.2 Variation in seed characteristics

5.2.1 Seed length and width

There were differences in seed length and width between provenances and between families within provenances (see Table 5.1). These differences were significant at the 1 % and 5 % levels of probability respectively. Mean family seed length ranged from 6.9 to 10.0 mm, with an overall mean of 8.5 mm, and family seed width from 4.7 to 6.7 mm, with an overall mean of 5.8 mm. Mean provenance seed length varied from 8.26 mm in Nimbin provenance (No. 2) to 8.94 mm in Mummulgum provenance (No. 9) (see Table 5.2).

Partitioning of total variation using components of variance revealed that differences between provenances accounted for 29.0 % of the total variance in seed width compared to families within provenances, which did account for 15.4 %.

5.2.2 Seed weight

There were significant differences between provenances in 25-seed weight at the 1% level of probability and between families-within-provenances at the 5% level (see Table 5.1). The coefficient of variation of 12.3% showed that seed weight was highly variable. The 25 seed-weight ranged from 0.19 g to 0.51 g, with an overall mean of 0.36 g. Mean provenance seed weight ranged from 0.25 g in the Porters gap provenance (No. 4) to 0.42 g in Mummulgum provenance (No. 9) (see Table 5.2).

Partitioning of the total variance in seed weight showed that the differences between provenances accounted for 39.4 % of the total variance while families within provenances did not account for any (Table 5.13).

Table 5.1. Summary of analyses of variance of seed weight, seed length and seed width. (** indicates significance at the 1 % level of probability; ns - not significant at the 5% level). prov=provenance. The numbers in brackets are the missing values.

Source of		Seed--Weight			Seed--Length			Seed--Width	
variation	DF	MS	F	DF	MS	F	DF	MS	F
Provenance	10	0.0285	16.05**	5	0.68357	6.94**	5	1.9224	15.77**
Family/prov.	24	0.0011	0.60 ns	18	1.67786	17.03**	18	0.3936	3.23**
Residual	144	0.00176		44(2)	0.09852		44(2)	0.1219	

Variation in seed size has been documented in other forest tree species and normally results in variation in seedling growth and vigour, with larger seeds producing larger and more vigorous seedlings. Seed size effects are normally strongest during the first growing season and diminish with age of the seedling (Shear and Perry, 1985). The variation in seed size is largely due to effects associated with the maternal tree, but it can also have a genetic as well as an environmental component associated with the mother tree (St. Clair and Adams, 1991). In Loblolly pine, female parent trees accounted for about 90 % of the total variation in seed size (Perry, 1976).

Results of simple regression analysis of seed weight on geographic locations and climatic factors of seed source showed a low but significant correlation with latitude (r = 0.61), longitude (r=0.57) and rainfall (r = 0.44). These relationships were significant at the 5% level of probability. The relationship with temperature was positive (r=0.34) but non-significant at the same level of probability (Table 5.12).

Table 5.2. Mean seed weight, length and width in eleven provenances of *Grevillea robusta*.

No./Provenance	Seed width (g)	Seed length (mm)	Seed weight (g)
1. Emu vale	-		0.34
2. Nimbin	5.58	8.26	0.36
3. Bunya mts.	-	-	0.37
4. Porters gap	-		0.25
5. Albert river	-	-	0.39
6. Wivenhoe	5.14	8.53	0.35
7. Rappville	5.90	8.58	0.40
8. Duck creek	6.18	8.53	0.41
9. Mummulgum	6.12	8.94	0.42
10. Mcphersons	-	-	0.41
11. Boyd river	-	-	0.35
Mean	**5.8**	**8.5**	**0.36**
CV (%)	**6.1**	**3.7**	**9.2**
LSD	**0.285**	**0.256**	**0.028**

Provenances from lower altitudes had significantly heavier seeds than those from higher altitudes, as suggested by the significant negative relationship between seed weight and altitude of seed source (r = -0.51). The statistical significance of the relationship between seed weight and altitude of seed source was largely due to the smaller size of seeds from the Porters gap provenance and larger size of seeds from the Mummulgum provenance. Correlation coefficients between seed weight, seed length and width and, geographic and environmental parameters of source are shown in Table 5.12. The relationship between seed weight and precipitation in the dry, wet, hot and cold quarters were low and non-significant. Regression analysis showed that the effects of altitude and temperature and longitude were highly significant in explaining variation in seed weight.

Similar studies on variation of seed weight along environmental gradients in a number of tree species have given conflicting results. For example, Baker (1972) found a significant decrease in average seed weight of species with increasing altitude, and suggested that shorter growing season characteristic of such environments selects for small seed size because of insufficient time for seed to develop. In contrast, McWilliams *et al.* (1968) found larger seed sizes of plants of *Amarunthus retroflexus* from higher altitudes compared to those from lower altitudes. They concluded that harsh environmental conditions at higher altitudes would favour production of larger seeds.

5.3 Variation in growth characteristics

The results of this study showed the existence of wide variations in seedling growth characteristics between and within provenances. Results of analyses of variance showed highly significant differences between provenances in all traits assessed in the study except specific leaf area. Most of the traits also showed significant family within provenance differences.

Block effects in analyses of variance in all traits were highly significant, indicating that the blocking structure used in the experiment was adequate in isolating and removing unwanted sources of variation.

Partitioning of total variance in each trait into components due to provenances, families within provenances and error showed widely differing proportions of variance accounted for by each source, depending on the trait. Generally, the main effects of provenances accounted for higher proportions of the total variance in different traits, from 3.1 % to 43.7 % (19 to 100 % using intraclass correlations), compared to families within provenances (see Table 5.13).

5.3.1 Variation in height growth

Assessments made at ages 1, 3, 5 and 6 months were used to study variation in height. Analyses of variance revealed highly significant provenance differences in height growth in all four assessments at the 1 % level of probability (see Table 5.3). Significant family-within-provenance differences existed at the ages of five and six months of age at the 5 % and 1 % levels of probability respectively, but differences at the ages of one and three months were not significant at the 5 % level. Table 5.4 summarises the height growth of the eleven provenances of *Grevillea robusta* used in the study, in order of decreasing height for all four assessments.

Table 5.3. Summary of analyses of variance of height growth in the eleven provenances of *Grevillea robusta* at the ages of 1, 3, 5, and 6 months. Degrees of freedom (df) are shown in parenthesis for each source of variation. (Prov = Provenance; Prov/fam = families within provenance). Significance levels of F-test ** - $p < 0.01$; *** - $p < 0.05$; ns - not significant at $p < 0.05$ level. Values in brackets following residual degrees of freedom (DF) are the number of missing values.

		Source of variation							
	Age (Months)	[Provenance (df=10)]			[Prov/fam (df=22]			[Residual]	
Trait		MS	F	p-value	MS	F	p-value	DF	MS
Height 1 [HT-1]	1	17.985	20.38**	< 0.01	1.0735	1.22 ns	0.235	263(1)	0.882
Height 2 [HT-2]	3	61.314	10.28**	< 0.01	8.374	1.40 ns	0.114	263(1)	5.966
Height 3 [HT-3]	5	83.389	5.37**	< 0.01	3.655	2.35**	< 0.01	263(1)	1.463
Height 4 [HT-4]	6	231.33	4.64**	< 0.01	125.85	2.63**	< 0.01	262(2)	47.76

Ranking of provenances for seedling height growth was consistent at both extremes, with some provenances clearly being among the best or the worst. Middle rankings were however not consistent. Rappville provenance (No. 7) was almost consistently the tallest, while Porters gap (No. 4) was the shortest in all assessments of height. LSD test separated the Porters gap (No. 4) and Bunya mountains (No. 3) provenances from all others (see Table 5.4).

The coefficient of variation (CV%) for height ranged from 13.6 % to 17.1 % in all four assessments. The coefficient of variation for final height was 12.3 %. However, partitioning of total variance showed a higher proportion of this variation to be accounted for by the differences between families within provenances (15.8 %) with provenances accounting for 7.1 % (Table 5.13).

Table 5.4. Mean height (HT) (cm) , least significant differences (LSD) and coefficients of variation (CV%) and provenance rankings of eleven provenances of *Grevillea robusta* at the ages of 1, 3, 5, and 6 months. For provenance identities, see Table 4.1.

	HT-1 (1 months)		HT-2 (3 months)		HT-3 (5 months)		HT-4 (6 months)	
Rank	Height	Prov	Height	Prov.	Height	Prov.	Height	Prov
1	6.89	8	17.12	8	28.22	9	49.38	7
2	6.88	7	17.07	9	27.65	7	44.14	5
3	6.65	9	16.06	5	27.12	10	43.28	8
4	6.35	1	15.91	1	26.85	8	43.16	9
5	6.16	5	15.88	7	25.31	5	43.12	11
6	6.01	10	15.33	10	24.47	11	42.70	10
7	5.51	6	14.48	11	23.93	6	41.36	1
8	5.40	11	13.97	6	23.01	2	39.03	2
9	4.84	2	13.28	2	22.75	1	37.14	3
10	5.02	3	12.81	3	19.77	3	36.98	6
11	4.26	4	11.17	4	16.32	4	33.93	4
LSD	0.53		1.54		2.21		3.91	
CV%	16.1		17.1		13.6		14.9	

Simple regression analysis of height growth on geographic location and environmental factors of the seed sources showed significant correlations with altitude and latitude of seed source. The correlation coefficients showed the strongest relationships with altitude, followed by latitude of seed source while the relationship with longitude was weak. Height growth was also significantly ($p<0.05$) correlated with temperature and rainfall of seed source (see Table 5.12).

At the end of the experiment, the relationship between altitude of seed source and height growth was negative and linear, suggesting a clinal pattern of variation in this trait ($r = -0.55$, see Figure 5.1). Provenances from higher altitude sources (eg., Bunya mountains (No. 3) and Porters gap (No 4) had lower mean height than the coastal lower altitude provenances (Figure 5.3 and Table 5.12).

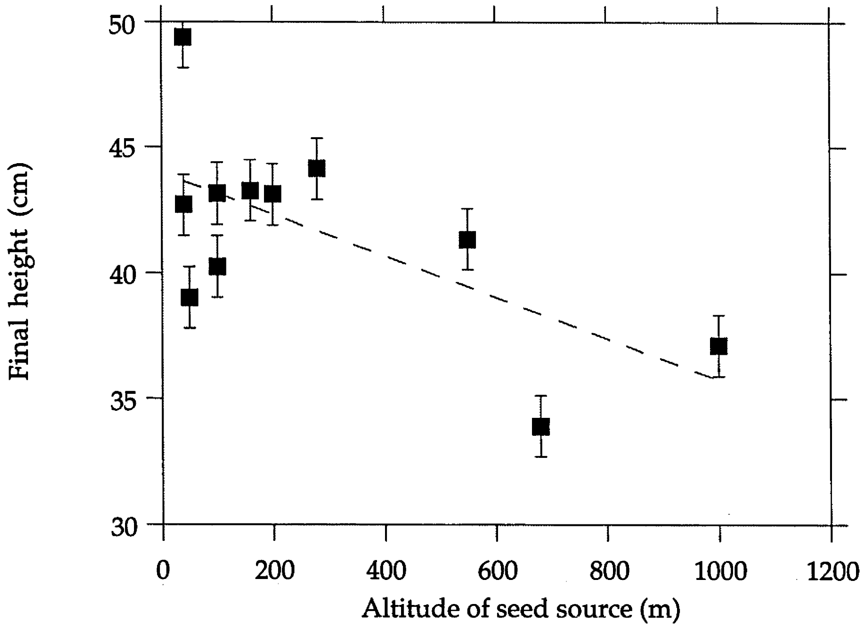


Fig. 5.1. The relationship between mean height growth and altitude of seed source of the eleven provenances of *Grevillea robusta* at age 6 months; (r=-0.55).

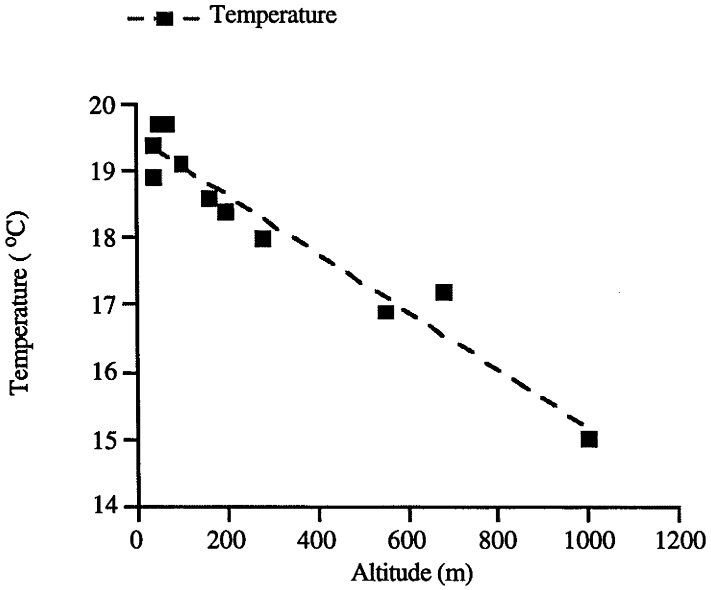


Fig.5.2. The relationship between altitude and the mean annual temperature of seed source of eleven provenances of G. *robusta* used in the study (r= -0.98).

However, the provenances from the lowest altitude were not necessarily the tallest. The tallest were from middle-lower altitudes (100 - 200 metres). The general relationship between height growth and altitude of seed source suggested that a factor or some factors of the seed source environment that change gradually with altitude, such as temperature or length of growing season may be important in explaining provenance variation in height growth. This assumption was supported by a strong negative relationship between temperature and altitude of seed source (r= -0.98, see Figure 5.2), suggesting that temperature of the seed source may play an important role in selection for height growth.

Correlation coefficient between longitude and final height growth was positive and significant (r = 0.57; $p<0.05$, see Table 5.12) presumably because rainfall decreases with increasing distance from the coast (Harwood, 1992). Moisture availability of the seed source environment may play an important role in determining the rate of height growth.

5.3.2 Variation in diameter growth

There were highly significant differences between provenances and between families within provenances in diameter growth at the 1 % level of probability at the ages of three and six months (Table 5.5). The coefficients of variation for diameter growth were low, 6.5 % and 5.5 % at 3 and at 6 months of age respectively. Mean diameter growth of seedlings at the end of the experiment ranged of 9.9 to 14.4 mm, with an overall mean of 12.6 mm. Provenance means ranged from 11.46 mm in Porters gap to 13.18 mm in McPhersons provenances. Table 5.6 shows the mean diameter of the provenances of *Grevillea robusta* at the ages of three and six months.

Correlations between mean diameter growth of provenances and the geographic location and climatic factors of seed source revealed a significant increase in diameter growth with increasing latitude, longitude, mean annual rainfall and temperature of seed source while diameter was found to decrease with increasing altitude of seed source.

Table 5.5. Summary of Analyses of variance of diameter growth of the 11 provenances of *Grevillea robusta* at 3 and 6 months. ** - significant at 1% and * - at 5% levels of probability respectively. (Degrees of freedom for each source of variation are shown in parenthesis). p-value is the probability value. Prov=provenance, fam=family.

Source of	Diameter 1 (3 moths)			Diameter 2 (6 months)		
Variation	MS	F	p-value	MS	F	p-value
Prov (10)	0.62757	7.88**	< 0.001	6.0402	12.73**	< 0.001
Prov/fam (22)	0.16025	2.01**	0.006	1.0747	2.26**	0.002
Residual (220 (4)	0.07965			0.4746		

Table. 5.6 Mean diameter growth of the eleven provenances of *Grevillea robusta* at 3 and 6 months of age. Least significant differences (LSD) and, coefficients of variation (CV%) are presented.

	Diameter 1 (3 months)		Diameter 2 (6 months)	
Rank	Mean (mm)	Provenance	Mean (mm)	Provenance
1	4.55	10 - (Mcphersons)	13.18	10 - (Mcphersons)
2	4.52	7 - (Rappville)	13.01	7 - (Rappville)
3	4.51	5 - (Albert river)	12.99	9 - (Mummulgum)
4	4.49	8 - (Duck creek)	12.77	8 - (Duck creek)
5	4.43	9 - (Mummulgum)	12.74	6 - (Wivenhoe)
6	4.39	1 - (Emu vale)	12.68	5 - (Albert river)
7	4.34	11 - (Boyd river)	12.55	2 - (Nimbin)
8	4.30	6 - (Wivenhoe)	12.54	11 - (Boyd river)
9	4.25	3 - (Bunya mts.)	12.24	1 - (Emu vale)
10	4.18	2 - (Nimbin)	11.98	3 - (Bunya mts.)
11	4.04	4 - (Porter's gap)	11.46	4 - (Porter's gap)
LSD	**0.16 mm**		**0.39 mm**	
CV%	**6.50**		**5.50**	

The correlations between final diameter and mean annual temperature, latitude and longitude of seed source were significant (r=0.70, r=0.84 and 0.63 respectively) (see Table 5.12). Seedlings from high altitude sources had smaller mean stem diameter than seedlings from lower altitude sources, as shown by the highly significant negative correlation between diameter and altitude of seed source (r= -0.84). Correlations with mean annual rainfall of seed source and precipitation in the dry, wet, hot, and cold quarters were low and non-significant.

The low correlation with mean annual rainfall of seed source implies that other factors that change with longitude other than moisture availability may be important. Figure 5.3 shows the relationships between diameter growth and altitude, latitude, mean annual temperature and mean annual rainfall of seed origin respectively.

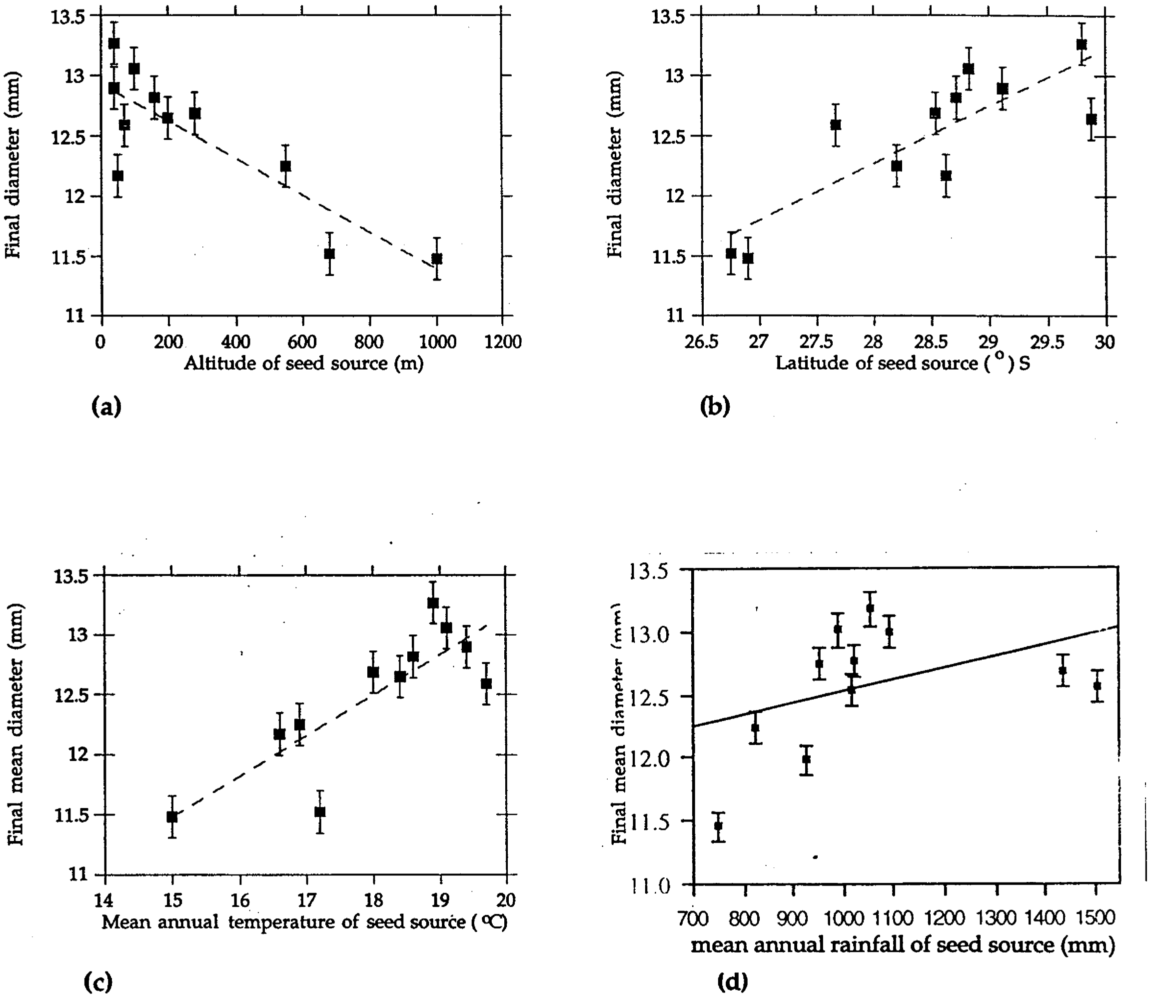


Fig. 5.3. Relationship between mean diameter and (a) altitude (r = -0.84), (b) latitude (r =0.84), (c) mean annual temperature (r = 0.70) and (d) mean annual rainfall (r =0.29) of seed source in eleven glasshouse grown provenances of *Grevillea robusta* at the age of six months. Vertical bars show the standard error of the mean.

Most of the variation in diameter growth was accounted for by differences between provenances, with 27.5 % of the total variance, whereas families-within-provenance variation accounted for 9.9 % (Table 5.13).

5.3.3 Variation in leaf growth characteristics

The amount of foliage in a plant can represent the plant's potential for photosynthesis and growth. Leaf area, leaf length and number of leaves were measured to assess variation in this potential. Other variables associated with leaf growth ie., specific leaf area and leaf to stem weight ratio were derived from the leaf area and leaf and stem dry weights. Leaf length and leaf area are a measure of leaf size, whereas specific leaf area gives an indication of cell size and shape.

Significant variation was observed among and within provenances in all leaf growth characteristics assessed or derived except specific leaf area. In most of these characteristics, provenance differences accounted for a higher proportion of the total variance compared to families within provenances.

Several studies of forest trees have found variation in leaf growth characteristics to have a strong genetic basis, while on the other hand, it has also been suggested that leaf growth in general and, leaf area in particular exhibit highly plastic responses to environmental conditions and show plastic responses under stress conditions such as moisture or fertiliser deficiency (Quisenberry, 1982). In this study, seedlings were grown under non-limiting conditions and this allowed expression of the seedlings' full potential for leaf growth characteristics.

5.3.3.1 Leaf area

Highly significant differences between provenances in leaf area were observed at the 1 % level of probability at two, four and six months of age. No significant differences between families within provenances were found at two and four months of age, but significant differences were detected at six months of age (Table 5.7). Leaf area of individual seedlings ranged from 1590 to 3093 cm^2 at the end of the experiment. Provenance mean leaf area ranged from 2696 cm^2 in the Porters gap (No. 4) to 3227 cm^2 in Mummulgum provenance (No. 9). The coefficient of variation for leaf area at the final harvest was 13.4 %. Table 5.8 summarises of mean leaf area of the provenances of *Grevillea robusta* used.

Table 5.7. Summary of analyses of variance of leaf growth characteristics in seedlings of *Grevillea robusta* at the ages of 2, 4 and 6 months. Significance of F test- ** - significant at 1% probability level ($p<0.001$); * - significant at 5% probability level ($p<0.05$) and, ns - not significant at 5% level ($p>0.05$).

		Source of variation							
	Age	[Provenance (df=10)]			[Family/Provenance (df=24)]			[Residual]	
Trait	(months)	MS	F	pvalue	MS	F	p-value	DF	MS
Leaf area 1	2	7102.8	16.53**	< 0.001	636.2	1.48 ns	0.100	93	429.6
Leaf area 2	4	153940	6.02**	< 0.001	2708	1.06 ns	0.407	89	25591
Leaf area 3	6	794398	5.07**	< 0.001	347303	2.21**	0.002	263	156825
Leaf length 1	2	11.445	11.99**	< 0.001	2.981	3.12**	< 0.001	224	0.954
Leaf length 2	4	58.969	10.70 **	<0.001	8.760	1.59*	0.050	220	5.513
Leaf Length 3	6	73.001	9.63**	< 0.001	17.480	2.31**	0.001	224	7.583

Table 5.8. Mean growth of leaf characteristics, least significant differences and coefficients of variation of 11 provenances (Prov.) of *Grevillea robusta*.

	Final leaf area		Final leaf length		
Rank	(cm^2)	Prov.	(mm)	Prov	Provenance
1	3227	9	40.34	4	Porters gap
2	3155	2	38.88	8	Duck creek
3	3130	10	38.61	2	Nimbin
4	3097	8	37.85	11	Boyd river
5	3055	6	37.28	10	Mcphersons
6	2977	7	37.26	5	Albert river
7	2887	5	36.95	1	Emu vale
8	2857	11	36.49	9	Mummulgum
9	3703	3	35.58	3	Bunya mountains
10	2673	1	35.47	6	Wivenhoe
11	2696	4	34.17	7	Rapville
LSD	201.1		3.2		
CV%	13.4		7.4		

Variation in leaf area was further partitioned into variation between provenances and between families within provenances using variance components for this trait (see section 4.5.3.2, Chapter 4). The results, presented in Table 5.13, showed that at the end of the experiment, provenances and families within provenances accounted for similar proportions of the total variance, 12.2 % and 11.6 % respectively.

Results of a simple regression analysis of final leaf area on geographic and climatic parameters of seed source showed a significant negative correlation with altitude of seed source (r = -0.84) and positive correlations with latitude (r =0.55), longitude (r = 0.64), rainfall (0.53) and temperature of seed source (r =0.81). The relationships between leaf area and altitude, latitude, temperature and mean annual rainfall of seed source are presented in Figure 5.4. Leaf area was also significantly correlated with mean annual maximum temperature (r=0.83), mean annual minimum temperature (r=0.72) and with precipitation in the wet and dry quarters of seed source. The relationships with latitude and mean annual rainfall and dry quarter precipitation of seed source were small and non-significant (Table 5.12).

5.3.3.2 Leaf length

Significant differences between provenances as well as between families within provenances in leaf length was detected at the 1 % level of probability (Table 5.7). At the end of the experiment, Porters gap (No. 4) had the longest mean leaf length (40.38 cm) while Rappville provenance (No. 7) had the shortest, 34.17 cm. The coefficient of variation was 9.3 %, and 10.2 % at four and six of months age.

Provenance variation accounted for a higher proportion of the total variance, with 19.3 %, compared to families within provenances which had 13.2 % (Table 5.13). The coefficient of variation in this trait was 7.4 %, lower than that of leaf area. Length of leaves was not significantly correlated with any of these parameters (Table 5.12).

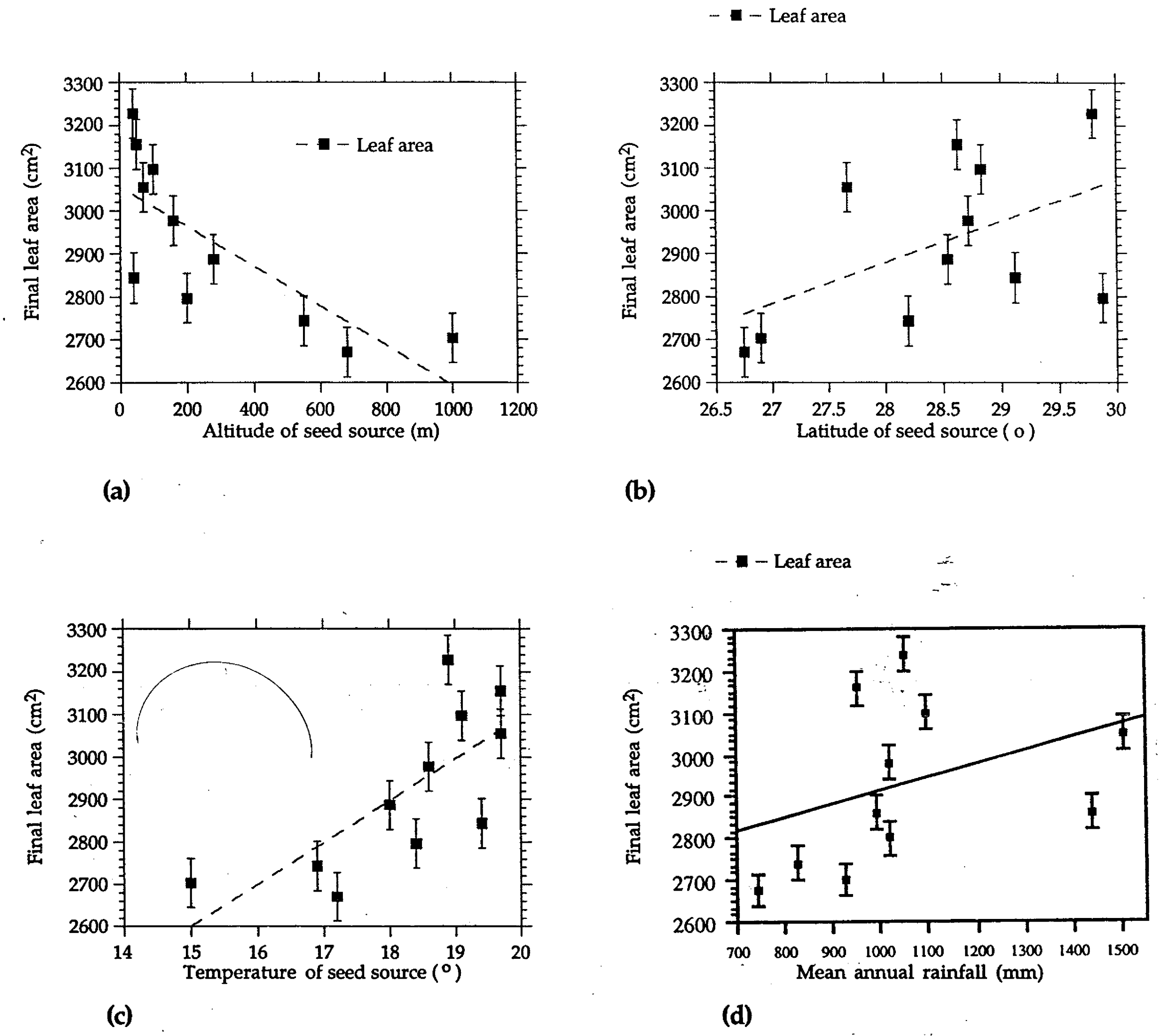


Fig. 5.4. The relationships between leaf area and (a) altitude (r=-0.84), (b) latitude (0.55), (c) Mean annual temperature (r=0.81) and (d) Mean annual rainfall (r=0.53) of seed origin of the eleven provenances of *Grevillea robusta*. The vertical bars show the standard errors of the mean.

5.3.4 Variation in mean internode length

Provenances as well as families within provenances differed significantly in mean internode length at the 1% level of probability (Table 5.9). Porters gap provenance (No. 4) had the shortest mean internode of 2.71 cm while Mummulgum (No. 9) had the longest, 4.41 cm (see Figure 5.5). The coefficient of variation for this trait was 15.1 %, indicating the trait is variable.

Families within provenances accounted for a higher proportion of the total variance in mean internode length with 17.3 % compared to provenances which accounted for only 4 % of the total variance (Table 5.13).

Table 5.9. Summary of analysis of variance of mean internode length at 6 months. Significance of F-test:-** - significant at the 1 % probability level. p-value - is the probability value. Degrees of freedom are shown in parenthesis.

Source of variation	MS	F	p-value
Provenance (10)	148.06	3.99**	<0.001
Family/provenance (22)	102.33	2.75**	<0.001
Residual (244(1))	37.15		

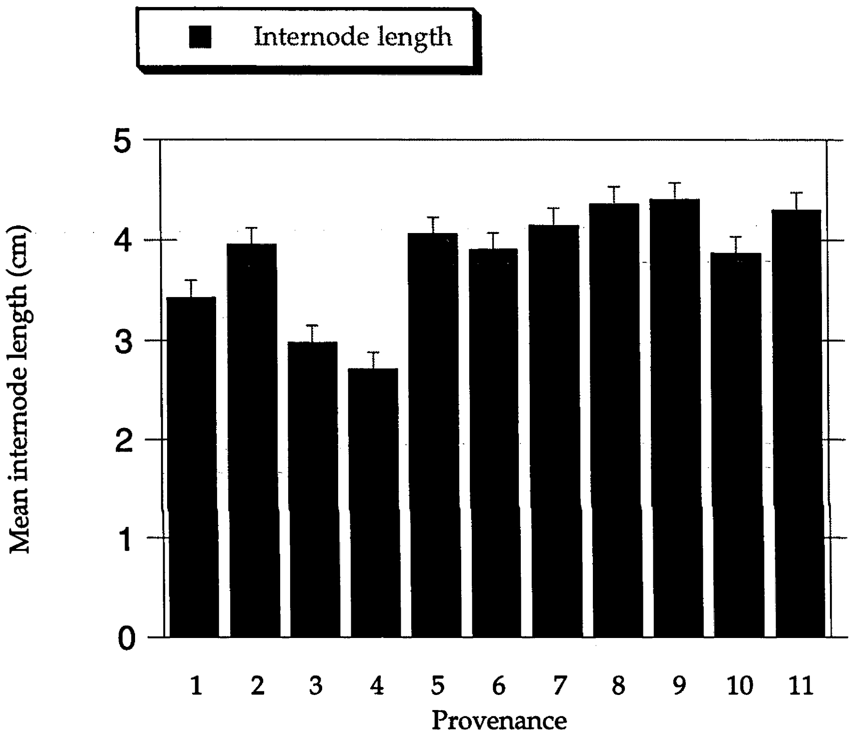


Fig. 5.5. Variation in mean internode length in the eleven provenances of *Grevillea robusta*. The vertical bars show the standard error of the mean. LSD = 0.41

Simple regression analysis revealed a decrease in mean internode length with increasing altitude of seed source and an increase with increasing latitude, longitude, mean annual rainfall and temperature of seed source These relationships are shown in Figure 5.6. Correlations with altitude (r= -0.83), latitude (r=0.81), longitude (r=0.75) and mean annual temperature (r= 0.72) of seed origin were significant at the 5 % level of probability. The mean internode length was positively correlated with precipitation in the hot quarter (r= 0.51) but correlations with rainfall of seed source and precipitation in the wet, dry and cold quarters were small and non-significant at the same level of probability (see Table 5.12).

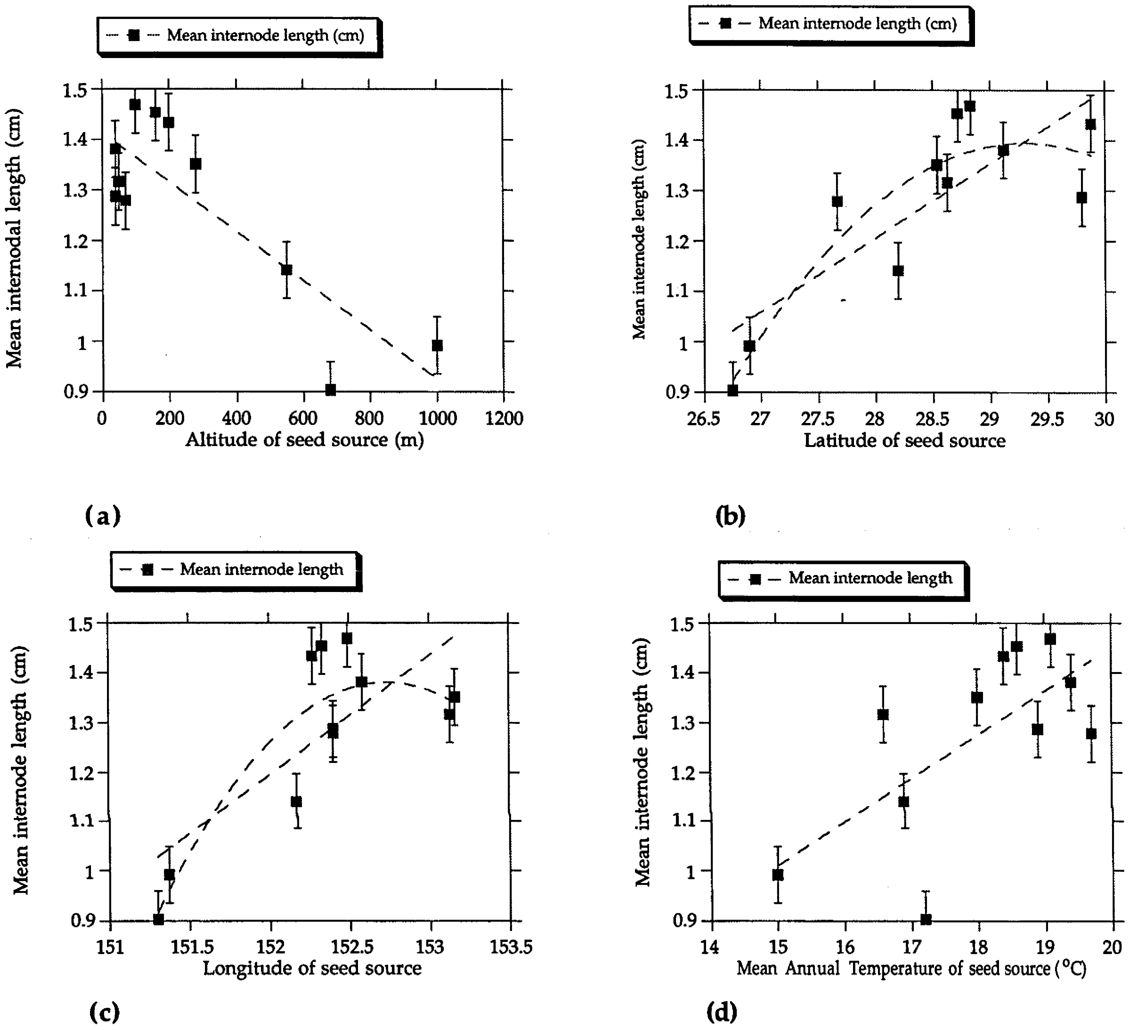


Fig. 5.6. The relationship between mean internode length and (a) altitude (r= -0.83), (b) latitude (r=0.81), (c) longitude (r= 0.75) and (d) mean annual temperature (r=0.72) of seed source of the eleven provenances of *Grevillea robusta*. The curvilinear relationships for latitude (r=0.91) and longitude (r=0.83) of seed source are shown. Vertical bars show the standard error of the mean.

5.3.5 Variation in biomass production

5.3.5.1 Shoot and root dry weights

There were significant differences between provenances in shoot dry weight at the 1% level of probability at the ages of two, four and six months and between families within provenances at the 5 % level at six months of age (see Table 5.10). No significant differences between families within provenances were detected at two and four months of age. At the end of the experiment, Rappville provenance (No. 7) had the highest shoot dry weight of 50.84 g. and Porter's gap (No. 4) the lowest of 42.26 g (Table 5.11).

Significant differences between provenances in root dry weight also were detected at the 1 % level of probability but the differences between families-within-provenances were not significant (Table 5.10). At the end of the experiment, Porter's gap provenance (No. 4) had the lowest mean root dry weight of 14.67 g. while Duck creek provenance (No. 8) had the highest, 18.54 g. The significant differences were due to low shoot dry weight in the Porters gap and high weights in the Duck creek (No. 8) and Rappville (No. 7) provenances (see Table 5.11).

5.3.5.2 Leaf and stem dry weights

Leaf dry weight at the end of the experiment ranged from 29.9 g. in the Porters gap provenance to 34.9 g in the Rappville provenance (Table 5.11). The differences between provenances and between families within provenances were highly significant at the 1 % level (see Table 5.10).

As with leaf dry weight, provenances differed significantly ($p<0.01$) in stem dry weight at the end of the experiment (Table 5.10), with Rappville provenance (No. 7) producing the highest stem biomass of 15.84 g and Porters gap producing the lowest, 12.32 g (Table 5.11). Families within provenances also differed significantly in stem biomass at the 5 % level of probability.

5.3.5.3 Root-to-shoot and leaf-to-stem dry weight ratios

No significant differences between provenances were detected in the shoot-to-root dry weight ratio at the end of the experiment. However, differences between families within provenances were strongly significant at the 1 % level of probability. The ratio ranged from 0.27 to 0.86 in all seedlings. Porters gap provenance (No. 4) allocated the highest amount of biomass to roots relative to shoots, resulting in the lowest value of the ratio, 0.50 and Duck creek provenance (No. 8) had the highest ratio of 0.57.

Simple regression analysis of shoot to root ratio at six months of age on geographic locations and climatic factors of seed source revealed no significant correlations except with the annual temperature range (r=0.57). The shoot to root dry weight ratio was negatively but not strongly correlated with altitude and the various measures of precipitation (see Table 5.12).

Significant differences between provenances as well as families within provenances were observed in leaf-to-stem dry weight ratio at the end of the experiment at the 1% level of probability (see Table 5.10). Mean provenance leaf-to-stem dry weight ratio ranged from 2.15 in the Albert river to 2.55 in Nimbin provenances (Table 5.11). No significant differences were detected between provenances and between families within provenances in leaf-to-stem dry weight ratio at the ages of six months.

5.3.5.4 Total biomass

There were differences between provenances as well as between families within provenances in seedling total biomass production at the 1% and 5 % levels of probability respectively (see Table 5.10). At the end of the experiment, mean total seedling biomass in all seedlings ranged from 57.8 to 68.5 g., with an overall mean of 64.6 g. Rappville provenance No. 7) produced the highest total biomass, while Porters gap (No. 4) produced the least (see Table 5.11). Ranking of the provenances in order of increasing total seedling biomass production and, using the LSD test showed that the first four provenances differed significantly from all the rest.

Simple regression analysis showed that total seedling biomass was significantly and positively correlated with latitude of seed source (r= 0.60) and negatively with altitude of seed source (r=-0.81, (log biomass, see Figure 5.7)). Seedling total biomass was weakly correlated with the mean annual temperature of seed source (r=0.32), but correlations with annual mean maximum temperature and annual temperature range were stronger and significant (r=0.45

and r=0.64 respectively). The relationship with mean minimum temperature was weak and non-significant (r= 0.12).

Table 5.10. Summary of analyses of variance of dry weight traits in eleven provenances of *Grevillea robusta* ages 2, 4 and 6 months. Significance levels for F-test ***- significant at 1 %, * - significant at 5 %, ns - not significant at $p<0.05$ probability level. [1](Error degrees of freedom vary because of the difference in number of seedlings harvested in the two harvests). The numbers following residual DF/ show the missing values.

	Source of variation							
	[Provenance (df=10)]			[Provenance/family (df=22)]			[Residual][1]	
Trait	MS	F	p-value	MS	F	p-value	DF	MS
Shoot weight 1	0.22848	8.73**	< 0.001	0.02070	0.79 ns	0.729	95/1	0.02618
Shoot weight 3	110.71	4.11**	< 0.001	44.62	1.62**	0.037	213/11	26.94
Root weight 1	0.00889	7.27**	< 0.001	0.00166	1.36 ns	0.155	96	0.001222
Root weight 3	35.523	4.97**	< 0.001	7.774	1.09 ns	0.360	259/4	7.14
Leaf weight 3	55.74	4.03**	<0.001	42.48	3.07**	<0.001	259/4	13.85
Stem weight 1	0.00870	10.7**	< 0.001	0.00088	1.08 ns	0.385	95/1	0.00081
Stem weight 3	24.029	4.94**	< 0.001	8.599	1.77**	0.021	259/4	4.866
Root : Shoot rto1	5794.6	9.44**	< 0.001	511.8	1.55 ns	0.077	96	0.00125
Root:Shoot rto 3	0.00995	1.29 ns	0.238	0.01517	1.96**	0.008	211/13	0.00772
Leaf:stem ratio	0.35085	3.53**	<0.001	0.35392	3.56**	<0.001	249/14	0.09929
Total biomass 1	0.32528	11.26**	< 0.001	0.03555	1.113 ns	0.326	95/1	0.03133
Total biomass 2	14.362	4.10**	< 0.001	2.755	0.79 ns	0.734	85/11	3.505
Total biomass 3	218.33	5.06**	< 0.001	78.32	1.810*	0.017	245	43.16

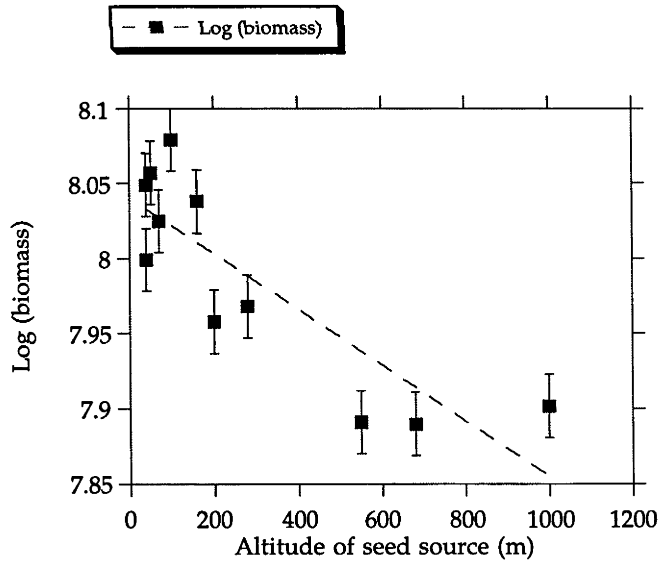


Fig. 5.7. The relationship between logarithm of biomass and altitude of seed source at the age of six months

Table 5.11. Biomass traits (dry weights), least significant differences (LSD) and coefficients of variation (CV%) of the 11 provenances (Prov.) of *Grevillea robusta* at 6 months of age.

	Shoot dry-wt (g)		Root dry -wt (g)		Root:shoot ratio		Stem-wt (g)		Total biomass (g)		Leaf dry weight (g)		Leaf to stem weightt ratio	
Rank	Mean	Prov	Mean	Prov.	Mean	Prov.	Mean	Prov	Mean	Prov	Mean	Prov	Mean	Prov
1	50.84	7	18.54	8	0.5683	8	15.84	7	68.45	7	34.96	7	2.55	2
2	48.87	6	18.53	11	0.5458	6	15.18	5	67.19	6	34.23	2	2.45	8
3	48.70	9	18.37	9	0.5447	11	15.03	10	67.06	9	34.09	6	2.42	3
4	48.17	3	18.35	6	0.5407	9	14.61	6	66.86	3	34.04	10	2.41	11
5	48.06	2	18.06	7	0.5320	3	14.55	9	65.19	11	33.75	3	2.40	4
6	46.99	10	17.91	3	0.5319	10	14.55	3	65.18	8	33.2	11	2.36	6
7	46.92	5	17.24	10	0.5235	7	13.95	1	64.41	10	33.05	5	2.34	11
8	46.89	11	16.59	5	0.5229	1	13.69	11	63.50	5	32.07	1	2.32	1
9	46.64	8	16.53	2	0.5188	5	13.59	8	63.12	2	31.7	7	2.25	7
10	46.01	1	16.50	1	0.5017	2	13.18	2	61.89	1	31.4	10	2.17	10
11	42.26	4	14.67	4	0.4973	4	12.32	4	57.76	4	29.85	4	2.15	5
LSD	2.94 g		1.51 g		0.0497 g		1.25 g		2.4 g		2.7 g		4.3	
CV %	8.5		12.2		6.8		10.4		8.0		15.1		17	

Fig. 5.12. Correlations coefficients for the relationships between geographical and environmental parameters of seed origin and seed and seedling growth traits in *Grevillea robusta*. Tests of significance were based 16 degrees of freedom. All correlations more than 0.305 are significant at the 5 % level of probability.

	Traits												
Variable	[SD-WT]	[HT -1]	[HT-2]	[HT-3]	[HT-4]	[DM-1]	[DM-2]	[LA-1]	[LA-2]	[LA-3]	LL-1	[LL-3]	[INT-L]
[Altitude]	- 0.512	- 0.343	- 0.522	- 0.782	- 0.552	- 0.476	- 0.843	0.557	- 0.587	- 0.839	- 0.610	0.250	-0.833
[latitude]	0.614	0.400	-0.621	0.800	0.762	0.694	0.838	0.707	0.522	0.551	0.806	- 0.096	0.809
[Longitude]	0.574	0.351	0.541	0.673	0.565	0.497	0.632	0.432	0.552	0.636	0.660	- 0.301	0.750
[Temperature]	0.339	0.205	0.349	0.361	0.389	0.265	0.703	0.387	0.465	0.813	0.436	- 0.236	0.719
[Rainfall]	0.443	- 0.011	0.223	0.372	0.277	0.225	0.288	0.080	0.476	0.527	0.449	- 0.206	0.508
[Cold Q ppt.]	0.330	- 0.158	- 0.522	0.238	0.205	0.140	0.177	- 0.034	0.381	0.396	0.415	- 0.029	0.344
[Hot Q ppt.]	0.448	0.049	0.295	0.368	0.238	0.262	0.291	0.123	0.453	0.486	0.448	- 0.125	0.514
[Wet Q. ppt.]	0.342	- 0.106	0.180	0.241	0.057	0.133	0.197	- 0.021	0.361	0.541	0.404	- 0.006	0.405
[Dry Q. ppt.]	0.288	- 0.173	0.024	0.124	0.136	0.130	0.045	- 0.083	0.325	0.226	0.378	0.011	0.212
[Mean max Temp]	0.383	0.392	0.505	0.669	0.424	0.292	0.680	0.486	0.506	0.830	0.504	- 0.201	0.800
[Mean Min Temp]	0.124	- 0.172	- 0.048	0.339	0.216	0.097	0.461	0.217	0.390	0.716	0.183	- 0.102	0.373
Annual T. range	0.439	0.583	0.658	0.651	0.411	0.366	0.605	0.620	0.512	0.696	0.504	- 0.053	0.742

	Traits											
	[SH-WT1]	SHTWT3	STWT3	[RT-WT 1	RTWT 3	[RT:SHT1]	[RT-SHT3]	[LF-DWT3]	[SLA-1]	[BM-1]	[BM-2]	[BM-3]
[Altitude]	- 0.437	- 0.461	- 0.302	- 0.374	- 0.384	0.442	- 0.261	- 0.365	- 0.123	- 0.469	- 0.424	- 0.371
[latitude]	0.614	0.370	0.366	0.618	·0.451	- 0.174	0.333	0.226	0.038	0.346	0.570	0.601
[Longitude]	0.315	0.482	0.379	0.324	0.198	- 0.149	0.012	0.356	- 0.062	0.393	0.523	0.265
[Temperature]	0.274	0.405	0.173	0.185	0.277	- 0.527	0.140	0.366	0.139	0.321	0.241	0.318
[Rainfall]	- 0.047	0.332	0.179	0.024	0.039	0.151	- 0.172	0.268	- 0.024	0.060	0.263	0.134
[Cold Q ppt.]	- 0.181	0.246	0.096	- 0.111	- 0.090	0.146	- 0.326	0.179	0.022	- 0.085	0.199	0.017
[Hot Q ppt.]	- 0.028	0.258	0.189	0.121	0.075	0.219	- 0.038	0.187	- 0.124	0.123	0.268	0.120
[Wet Q. ppt.]	- 0.121	0.110	0.028	- 0.028	- 0.030	0.164	- 0.092	0.069	- 0.176	- 0.038	0.160	- 0.020
[Dry Q. ppt.]	- 0.213	0.159	0.090	- 0.110	- 0.151	0.301	- 0.333	0.081	- 0.031	- 0.120	0.201	- 0.052
[Mean max Temp]	0.456	0.337	0.055	0.336	0.432	- 0.636	0.378	0.445	0.419	0.505	0.344	0.452
[Mean Min Temp]	- 0.110	0.345	0.248	- 0.133	- 0.008	- 0.208	- 0.275	0.206	0.186	- 0.079	- 0.014	0.123
Annual T. range	0.600	0.292	0.016	0.479	0.487	- 0.611	0.566	0.394	0.018	0.338	0.453	0.641

Table 5.13. Components of variance (Comp.) and partitioning of variation between provenance, between families within provenances and error and intraclass correlations for traits assessed in *Grevillea robusta*. The values in brackets show the components in percentage.

	Components of Variance						Intraclass Correlations	
Trait	Provenances Comp.	(%)	Families Comp.	(%)	Error Comp.	(%)	Provenance (%)	Families (%)
Seed weight	0.002	(39.4)	0	(0.0)	0.001	(60.6)	100	0.0
seed width	0.0637	(29.0)	0.0339	(15.4)	0.1219	(77.0)	65.3	34.7
Height 1	0.705	(43.7)	0.024	(1.5)	0.882	(54.8)	96.7	3.3
Height 2	2.206	(26.0)	0.301	(3.6)	5.966	(70.4)	87.9	12.1
Height 3	19515.7	(10.1)	27402.7	(14.2)	146289	(75.7)	41.6	58.4
Height 4	4.395	(7.1)	9.761	(15.8)	47.760	(77.1)	31.1	68.9
Diameter 1	0.019	(17.8)	0.010	(9.2)	0.080	(73.0)	65.9	34.1
Diameter 2	0.208	(27.5)	0.075	(9.9)	0.475	(62.6)	73.5	26.5
Leaf area 1	269.44	(37.1)	25.83	(3.6)	429.6	(59.3)	91.2	8.8
Leaf area 2	5285.87	(17.0)	186.0	(0.6)	25591	(82.4)	96.6	3.4
Leaf area 3	25163.3	(12.2)	23809.7	(11.6)	156825	(76.2)	51.4	48.6
Leaf length 3	2.102	(19.3)	1.441	(13.2)	7.363	(67.5)	59.3	40.7
Mean internode length	1.905	(4.0)	8.147	(17.3)	37.15	(78.7)	19.0	81.0
Shoot Weight 3	0.0008	(3.3)	0	(0.0)	0.0262	(96.7)	100	0.0
Shoot Weight 3	2.754	(8.6)	2.21	(6.9)	26.94	(84.5)	55.5	44.5
Root weight 1	0.0003	(19.7)	0.000005	(0.3)	0.0012	(80.0)	98.4	1.6
Root weight 3	1.156	(13.8)	0.0793	(1.0)	7.14	(85.2)	93.6	6.4
Stem weight 3	0.643	(10.8)	0.467	(7.8)	4.867	(81.4)	57.9	42.1
Leaf dry weight	0.5525	(3.1)	3.579	(19.9)	13.85	(77.0)	13.4	86.6
Biomass 1	0.0121	(27.5)	0.00053	(1.2)	0.0313	(71.3)	95.8	4.2
Biomass 2	0.484	(12.1)	0	(0.0)	3.505	(87.9)	100	0.0
Biomass 3	5.833	(10.9)	4.395	(8.2)	43.16	(80.8)	57.0	43.0

5.4 Patterns of variation in seed and seedling growth characteristics: Results of Principal Component Analysis (PCA)

Principal Component Analysis was carried out to study the patterns of variation in provenances of *G. robusta* on the basis of all traits combined together. The traits and the geographic locations and climatic parameters of seed origin were combined into a reduced set of four principal components (see Chapter 4, section 4.5.4 for procedures).

The first four principal components together accounted for about 88 % of the total variation in the original data set and therefore provided an adequate summary of the original information. The results of the analysis are given in Table 5.14, which shows the latent roots, the percentage of total variance explained by each component and the principal component loadings for all variables included. The first five variables with the highest loadings in each component are underlined.

The results showed that the first two components together accounted for 68.61 % of the total variance. The first principal component accounted for 52.2 % of the total variance (see Table 5.14 for loadings) and separated the provenances on the basis of growth characteristics, ie., leaf area, leaf dry weight, diameter growth and internode length. With heavy loadings on leaf growth characteristics, the component represented size, growth and vigour of seedlings as determined mainly by leaf area.

The second component, accounted for 16.11 % of the variance and was characterised by large positive loadings for altitude, temperature and rainfall of seed source and was interpreted to represent geographic origin and associated environmental factors of seed origin of *G. robusta*. This points to the importance of geographic and climatic parameters of seed source in determining variation.

The third and the fourth components accounted for 11.11 % and 9.11 % of the total variance. The third represented growth traits of height and diameter while the fourth represented a contrast between root growth characteristics, ie., root dry weight, shoot to root dry weight ratio and rainfall of seed source. This component was interpreted as representing adaptive characteristics in *G. robusta* as related to the seed source environment.

Table 5.14. Principal components (PC) showing loadings after orthogonal (varimax) rotation for seed and seedling growth traits, and geographic and climatic variables. on the first four components in *Grevillea robusta*. The first four components (PC-1 to PC-4) are given.

	Principal Components			
Trait	PC - 1	PC - 2	PC - 3	PC - 4
Height	0.0226	-0.1018	0.3667	0.0285
Diameter	-0.2512	0.1031	0.3165	-0.1322
Leaf area	- 0.3775	-0.0627	-0.0781	-0.0046
Leaf length	-0.1571	0.3417	-0.0217	0.0792
Internode length	-0.3417	-0.0217	0.0792	-0.1156
Stem dry weight	0.1599	-0.0496	0.4320	0.0763
Leaf dry weight	-0.4724	-0.0830	-0.2062	-0.0230
Root dry weight	-0.0952	-0.2326	-0.0045	-0.4166
Shoot dry weight	-0.0178	-0.0444	0.0425	0.0563
Root:shoot ratio	-0.1113	0.0080	0.0353	-0.6013
Leaf to stem ratio	-0.1793	-0.1165	-0.6158	-0.1285
Total biomass	-0.3880	-0.0036	0.0356	-0.1909
Latitude	0.1354	0.2832	0.2395	-0.0930
Altitude	-0.0434	0.3945	-0.0534	-0.0511
Longitude	-0.0829	-0.2947	0.0826	0.3528
Rainfall	-0.2517	-0.1356	-0.0616	0.4434
Temperature	0.0321	0.4196	-0.0416	-0.0929
% of Total Variance accounted for.	52.20	16.41	11.11	9.11
Cumulative %	59.20	68.61	78.72	83.83

To illustrate the patterns of variation, the first two components were plotted (Figure 5.8). The figure shows a separation of provenances into two major groups. The first group was composed of Mcphersons, Albert river, Duck creek, Boyd river, Mummulgum, Rapville and Wivenhoe provenances which clustered together. The second group was more divergent and consisted of Porters gap, Emu vale and Bunya mountains provenances. Nimbin provenance substantially deviated and formed a group of its own. A look at the plot revealed that the provenances were grouped on the basis of altitude of seed source and to some extent on the basis of the habitat type. Principal component analysis revealed a similarity between groups of provenances from the lower altitudes and higher altitude sources.

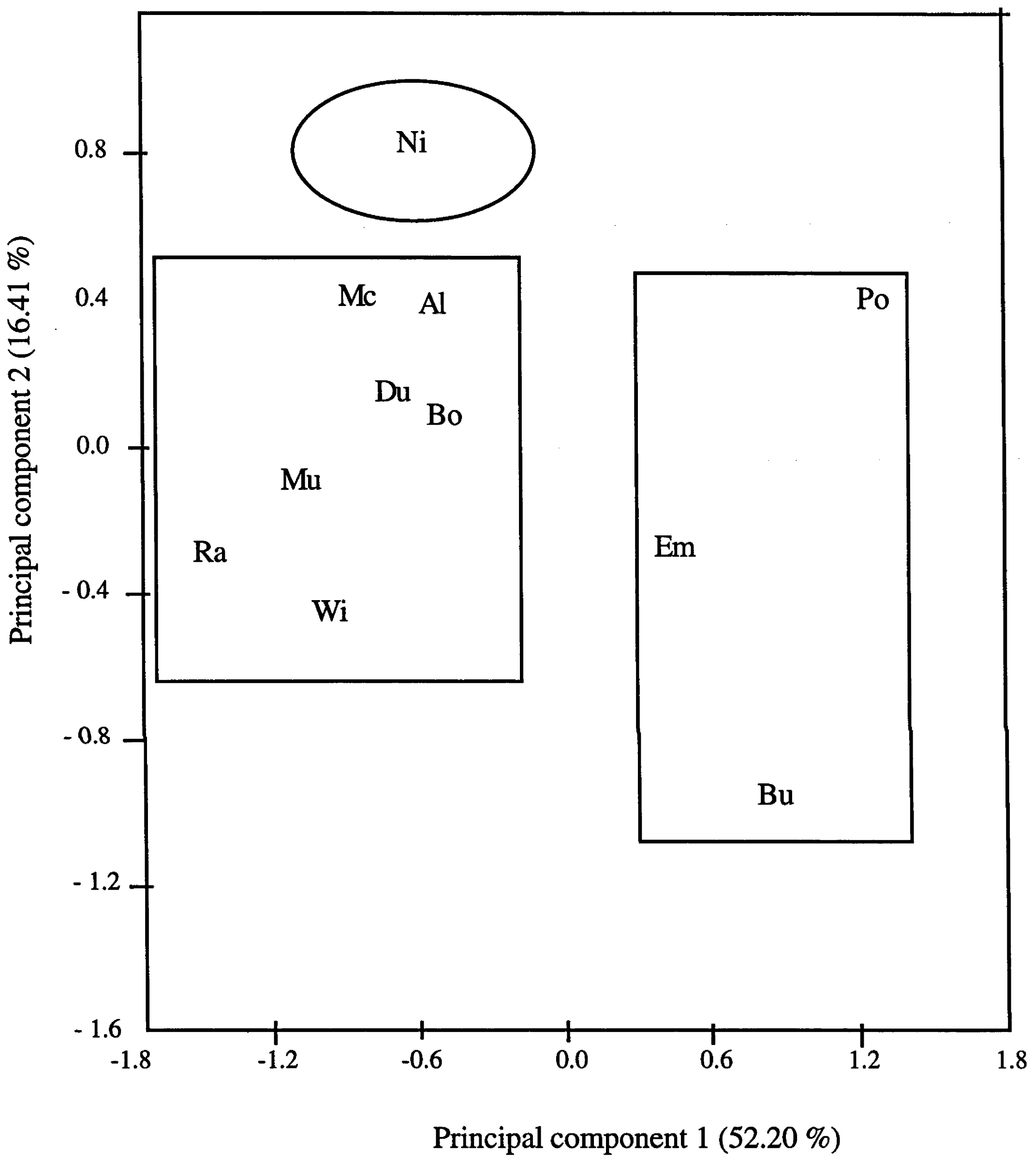


Fig. 5.8. Plot of the relationship between the first and the second principal components in seedlings of *Grevillea robus*ta. Mcphersons (Mc), Nimbin (Ni), Albert river (Al), Duck creek (Du), Boyd river (Bo), Mummulgum (Mu), Rapville (Ra), Wivenhoe (Wi), Porters gap (Po), Emu vale (Em) and Bunya mountains (Bu) are the provenances shown above.

The inverse relationships between growth traits and altitude of seed source emphasised the effect of altitude of seed source on growth of traits of seedlings of *G. robusta.* A plot of the first component and altitude of seed source showed that as the altitude of seed source increased, the value of each component decreased, suggesting that as the altitude of seed source increased, growth and size of seedlings decreased (Figure 5.9). Note that a positive component was associated with smaller seedling sizes and a negative component with larger sizes.

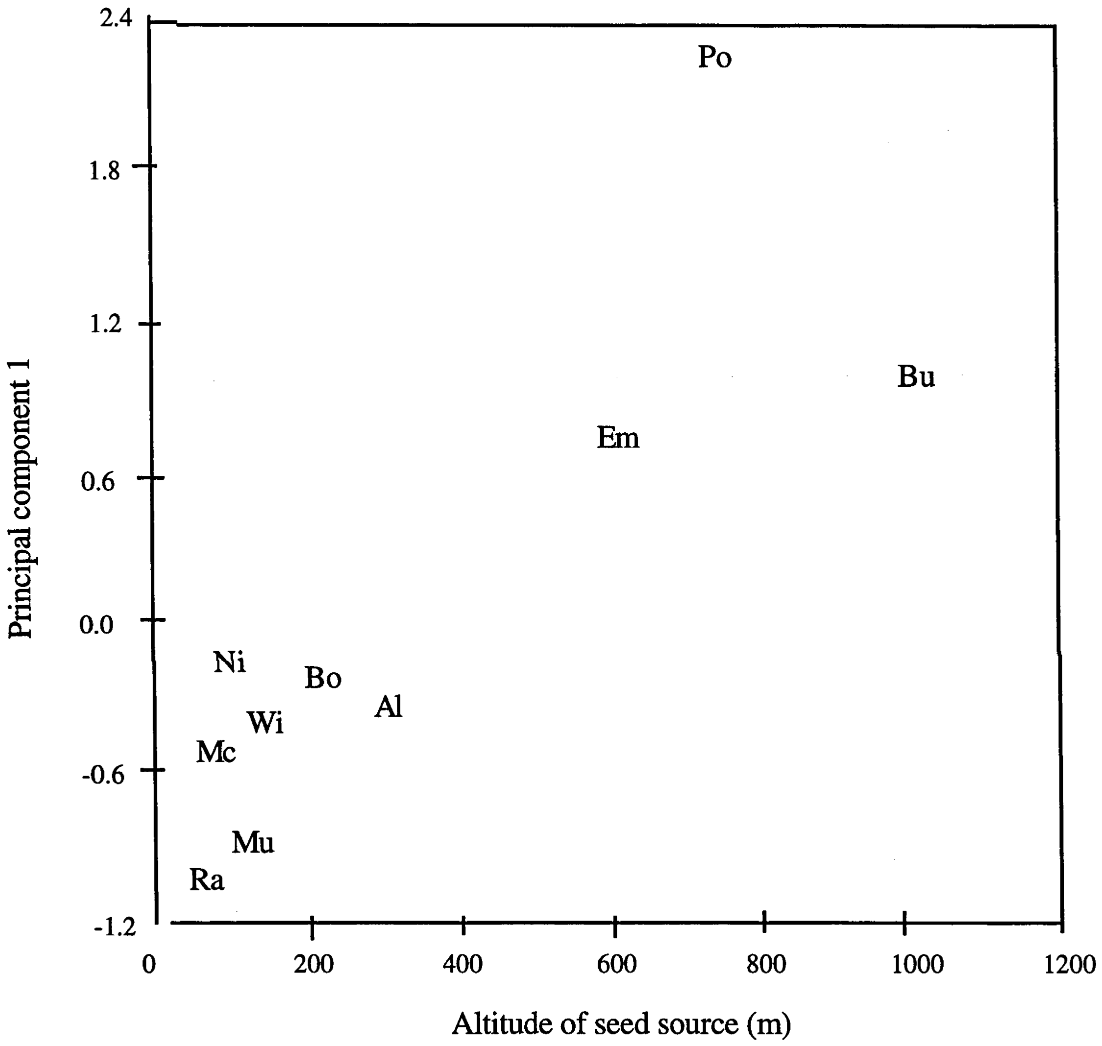


Fig. 5.9. The relationship between the first principal component and altitude of seed source in *Grevillea* robusta using provenance labels. The negative values of the principal component represent the faster growing provenances. The provenances shown are: Em- Emu vale, Ni-Nimbin, Bu- Bunya mountains, Po- Porters gap, Al- Albert river, Ra- Rappville, Mu- Mummulgum, Bo- Boyd river, Wi- Wivenhoe, Mc-McPhersons. (nb Duck Creek data not available)

CHAPTER 6

ESTIMATES OF HERITABILITY IN AND CORRELATIONS BETWEEN SEED AND SEEDLING GROWTH TRAITS IN *GREVILLEA ROBUSTA*

6.1 Introduction

Estimates of family and individual tree heritability and phenotypic correlations among seed and seedling growth traits are presented in this chapter. Heritability estimates indicate the strength of inheritance and can be used to predict the likely genetic gain in a breeding programme. On the other hand, tree breeding methods and possibilities of improvement may depend also on the magnitude of the correlation between traits of interest (Morgenstern 1969). The importance of these values in breeding of *Grevillea robusta* is discussed.

6.2 Estimates of trait heritability

6.2.1 Family and individual tree heritability

Heritability estimates were calculated using the methods described in Chapter 4 using variance components for each trait, assuming the provenances and families within provenances were random effects. In the estimation of heritability, it was assumed a half-sib relationship existed within families. The genotypic variance due to half-sibs is equivalent to one quarter of the total genetic variance (Falconer, 1989)

The estimates of family and individual tree heritability of traits varied widely among the traits. The results are presented in Table 6.1. Heritability estimates reported here represent variation within provenances and have no relationship with differentiation between provenances.

In all traits assessed, family heritability was larger than individual tree heritability. For example, diameter, height and leaf traits had generally high heritability estimates while mean internode length had somewhat low estimates. Height growth at the age of five months showed moderate estimates, having almost equal proportions of genotypic to phenotypic variance ($h^2 = 0.50$ and $h^2 = 0.40$ for family within provenance and individual tree among family in provenance heritability). The estimates for height seemed to decrease with age, with a relatively lower value of 0.34 at the age of six months.

Some traits had heritability values greater than unity; eg., seed weight, diameter at six months (1.61), leaf area at two months of age (2.33) and leaf length (1.05) (see Table 6.1). However, leaf length had individual tree heritability of less than one. By definition, heritability values cannot be greater than one (Zobel and Talbert, 1984). A greater value is an overestimate and emphasises the imprecision of heritability estimates. The high estimates obtained in this study, particularly in leaf area, diameter and biomass traits may be associated with difficulties in measuring these traits and the resulting errors and may also indicate unreliability of the individual tree level estimates. McKeand (1978), cited in Zobel and Talbert, (1984) also obtained a heritability of 1.25 in an analysis of half-sib progeny tests in Black walnut (*Juglans nigra*). High estimates of heritability are likely to be obtained in a glasshouse because of the reduced environmental variation in the glasshouse (Zobel and Talbert, 1984).

With the exception of the heritability values greater than one, most traits showed high heritability. The lowest value of 0.19 was obtained in mean internode length and the highest, 0.91 in root dry weight at the age of two months. The higher family within provenance estimates compared to the individual tree estimates may be consistent with the significance and importance of the provenance effects for the respective traits (see Chapter 5).

Heritability of provenance effects was not estimated for the traits assessed in the study because provenance heritability does not reflect the amount of genetic gain that can be achieved in an improvement programme through provenance selection (Johnson *et al.*, as cited in Otegebeye and Samarawira, 1992).

Table 6.1. Estimates of heritability in seed and seedling traits of glasshouse grown seedlings of *Grevillea robusta*.

Trait	Family heritability (h^2_f)	Individual tree heritability (h^2_i)
Seed weight	2.45	1.57
Seed width	1.83	1.37
Height 1	2.95	1.75
Height 2	1.41	1.04
Height 3	0.50	0.40
Diameter 1	0.92	0.71
Diameter 2	1.61	1.10
Internode length	0.19	0.16
Leaf area 1	2.33	1.49
Leaf area 2	0.81	0.68
Final Leaf area	0.60	0.49
Final Leaf length	1.05	0.89
Shoot dry weight	0.14	0.13
Shoot dry weight	0.39	0.35
Root dry weight 1	0.96	0.79
Root dry weight 3	0.64	0.55
Leaf dry weight	0.15	0.12
Stem dry weight 3	0.51	0.43
Total biomass 1	1.48	1.10
Total biomass 2	0.55	0.49
Total biomass 3	0.52	0.44

6.3 Correlations among seed and seedling growth traits

Phenotypic correlations were used to study the degree of correlations between various traits assessed. Phenotypic correlations may, at least sometimes, provide accurate approximation to underlying genetic correlations among traits(Lofsvold, 1986). A matrix of phenotypic correlations, based on provenance mean values for seed and seedling growth traits assessed in the study is given in Table 6.2. Of the 324 possible correlations, 196 were significant at the 5 % level of probability.

Seed weight was significantly correlated with all growth traits except leaf length, root to shoot dry weight and leaf to stem ratios. Highest correlations obtained were with leaf area at four months (0.89), height at five months (r=0.88), diameter at three months (r= 0.83) and total seedling dry weight at four and six months of age (r= 0.81 and r= 0.84 respectively). Correlations of seed weight with seedling traits were all positive except with leaf length (r= -0.49). It has been shown that seed size affects initial seedling size and, therefore, a wide variety of size-related traits in young plants. Seed size affects plant size as well as reproductive effort in later life of the plant by setting off a chain of correlations. For example, seed weight correlates positively with seedling size and seedling size correlates positively with adult with size and reproductive effort (Harper *et al.*, 1970; Chapin III *et al.*, 1993).

Correlations between leaf length and most growth traits showed that seedlings that had longer leaves were slower growing than those with short leaves as revealed by negative but non-significant relationships between leaf length and most growth traits (see Table 6.2). There was no significant relationship between length and area of leaves but significant negative correlations existed between leaf length and stem and leaf dry weights (r= -0.75 and -0.71 respectively, p<0.05).

The correlations between traits in this study suggest that it may be possible to genetically improve a number of traits simultaneously *G. robusta*, if such correlations reflect underlying genetic correlations. A strong and significant correlation between height and mean internode length emphasised the importance of internode length in determining height and the possibility of complementary improvement. Although both the number and length of internodes may contribute to total height increment, they may vary independently among trees (Cannel *et el.*, 1976). These correlations suggest that it is possible to genetically recombine two such traits to produce progeny with superior growth.

This is important in particular where the selected traits possess high heritability. For example high correlations among traits such as leaf area, height, diameter and stem dry weight (Table 6.2), and the high heritabilities of these traits suggest that these traits can be improved simultaneously, with possibilities for high gains. Height was also highly correlated with most growth traits.

Table 6.2. Martix of correlation coefficients between seed and seedling growth traits in glasshouse grown seedlings of *Grevillea robusta*.

TRAIT																										
[HT-1]	1.000																									
[HT-2]	0.901	1.000																								
[HT-3]	0.757	0.884	1.000																							
[HT-4]	0.772	0.795	0.837	1.000																						
[DM-1]	0.804	0.889	0.868	0.863	1.000																					
[DM-2]	0.615	0.804	0.947	0.748	0.826	1.000																				
[LA-1]	0.832	0.888	0.834	0.887	0.894	0.806	1.000																			
[LA-2]	0.565	0.621	0.787	0.574	0.604	0.664	0.604	1.000																		
[LA-3]	0.286	0.485	0.743	0.356	0.395	0.727	0.386	0.761	1.000																	
[LL-1]	0.513	0.717	0.704	0.665	0.705	0.698	0.778	0.566	0.423	1.000																
[LL-2]	-0.113	0.102	-0.037	-0.055	-0.107	-0.045	0.027	-0.048	0.071	0.543	1.000															
[LL-3]	-0.481	-0.331	-0.432	-0.361	-0.314	-0.283	-0.100	-0.276	-0.217	0.108	0.443	1.000														
[BM-1]	0.850	0.872	0.761	0.867	0.828	0.702	0.977	0.519	0.298	0.715	0.059	-0.088	1.000													
[BM-2]	0.858	0.831	0.787	0.739	0.839	0.642	0.783	0.766	0.385	0.741	0.093	-0.402	0.731	1.000												
[BM-3]	0.576	0.457	0.653	0.511	0.481	0.806	0.383	0.675	0.493	0.186	-0.433	-0.767	0.334	0.624	1.000											
[STW-3]	0.618	0.536	0.653	0.672	0.746	0.570	0.492	0.561	0.260	0.201	-0.539	-0.754	0.431	0.620	0.710	1.000										
[LF DWT]	0.446	0.287	0.474	0.383	0.178	0.244	0.178	0.529	0.465	0.113	-0.145	-0.714	0.184	0.492	0.864	0.424	1.000									
[SHTWT-1]	0.854	0.885	0.763	0.845	0.849	0.731	0.977	0.493	0.258	0.692	-0.014	-0.116	0.983	0.718	0.341	0.455	0.133	1.000								
[SHTWT-3]	0.566	0.439	0.659	0.576	0.468	0.469	0.340	0.672	0.518	0.193	-0.325	-0.838	0.301	0.630	0.929	0.756	0.905	0.270	1.000							
[RTW-1]	0.808	0.868	0.749	0.845	0.895	0.719	0.959	0.480	0.209	0.714	-0.022	-0.118	0.954	0.728	0.344	0.523	0.076	0.976	0.258	1.000						
[RTWT-3]	0.588	0.549	0.671	0.477	0.499	0.537	0.500	0.625	0.477	0.397	-0.222	-0.541	0.443	0.665	0.904	0.495	0.738	0.477	0.728	0.479	1.000					
[RT:SHT-1]	-0.115	-0.615	-0.128	0.031	0.147	-0.212	-0.143	0.113	-0.313	-0.165	-0.364	-0.220	-0.153	0.045	0.237	0.458	-0.011	-0.148	0.170	0.045	0.104	1.000				
[RT:SHT-3]	0.577	0.591	0.555	0.310	0.501	0.503	0.578	0.519	0.352	0.481	-0.089	-0.175	0.523	0.620	0.599	0.256	0.349	0.583	0.327	0.588	0.853	0.009	1.000			
[LF:STRTO]	-0.352	-0.409	-0.401	-0.484	-0.666	0.509	-0.438	-0.156	0.013	-0.173	0.409	0.285	-0.375	-0.273	-0.078	-0.722	0.291	-0.439	-0.125	-0.527	0.047	0.340	0.021	1.000		
[INT.L]	0.594	0.777	0.905	0.740	0.667	0.851	0.738	0.671	0.750	0.782	0.251	-0.300	0.693	0.668	0.567	0.391	0.515	0.670	0.565	0.651	0.675	-0.224	0.555	-0.118	1.000	
[SD-WT]	0.689	0.790	0.887	0.701	0.833	0.753	0.663	0.894	0.683	0.565	-0.121	-0.485	0.586	0.808	0.743	0.713	0.523	0.580	0.717	0.622	0.706	0.199	0.562	-0.339	0.738	1.000
	[HT-1]	[HT-2]	[HT-3]	[HT-4]	[DM- 1]	[DM-2]	[LA-1]	[LA-2]	[LA-3]	[LL-1]	[LL-2]	[LL-3]	[BM-1]	[BM-2]	[BM-3]	[STWT-3]	[LFD-WT]	[SHTWT-1]	[SHTWT-3]	[RTWT-1]	[RTWT-3]	[RT:SHT-1]	[RT:SHT3]	[LF:ST-RTO]	[NTN.L]	[SD-WT]

CHAPTER 7

GENERAL DISCUSSIONS AND CONCLUSIONS TO THE STUDY OF VARIATION IN SEED AND SEEDLING GROWTH CHARACTERISTICS IN *GREVILLEA ROBUSTA*

7.1 Introduction

This study revealed significant levels of variation between provenances of *Grevillea robusta* in all seed and seedling growth traits except specific leaf area. Provenance variation within the species' natural range was expected considering the wide environmental differences throughout its natural range. Families within provenances also differed substantially in some traits. The study showed that on average, most of the variation in *G. robusta* was mainly due to provenance variance compared to families within provenance variance. Seed and most seedling growth traits were significantly correlated with the geographic location and climatic factors of seed source, with altitude showing the strongest effects.

Several growth traits showed high estimates of heritability, suggesting stronger genetic than environmental effects in these traits. Since response to selection is proportional to heritability of a trait, the high proportions of genetic variance in most traits suggested the potential for successful genetic improvement in *G. robusta.*

7.2 Variation and partitioning of variation among and within provenances in seed characteristics and growth

Previous studies using protein electrophoresis (Harwood, 1992), growth and morphological characteristics (Kalinganire and Hall, 1993) (see Chapter 3) revealed significant variability among some natural populations of *Grevillea robusta.* These studies confirmed the existence of significant variation between provenances and between families within provenances in the species. The

Rappville, Duck creek and Mummulgum provenances were almost consistently the best in growth for most traits whereas Porters gap was consistently the worst.

The distribution and patterns of variation among or within plant populations depends not only on the species characteristics and other stochastic factors, but also on the type of traits studied. For example, in Lodgepole pine (*Pinus contorta*), 6 % of the total isozyme variation among populations, whereas 38 % of the total morphological variation is found among populations (Wheeler and Gurries, 1982).

Variation in *G. robusta* was found to be primarily due to differences between provenances, although a large within provenance component was also detected. However, the distribution of variation among and within populations of the species varied considerably among traits. For example, differences among provenances accounted for most of the variation in diameter growth, leaf area and leaf length and dry weight traits. In contrast, family within provenance variation accounted for most of the variation in height and mean internode length.

The general observation is that may be possible to obtain genetic gains in *G. robusta* through both mass and family selection. This is because genetic improvement and methods of selection depend on the distribution of variation within a species. For example, this study showed that selection for height growth in *G. robusta* would best be made through family selection. This is also true for diameter which had a considerable family within provenance component of variance.

Variation within plant populations has been less intensively studied because most studies concerned with patterns of genetic variation use provenance trials. Genetic variation within populations often occur in patches (Linhart, 1989) and is pronounced for many growth traits (Conkle, 1973; Namkoong and Conkle, 1976), allozymes (Linhart *et al.*, 1981) and adaptation to biotic and abiotic factors (Rehfeldt, 1993). Such studies in some species have documented extensive population differentiation, much of which has been interpreted as adaptation to heterogeneous environments.

7.3 Variation in relation to geographic location and climatic parameters of seed origin

Most seedling growth traits were significantly correlated with geographic and climatic parameters of seed source. Because inter-correlations among some traits were strong, similar relationships with geographic location and climatic parameters of seed source were evident in these traits. The relationships showed that altitude and latitude of seed source were important factors in explaining variation in growth. The relationships between growth and longitude were also important and may be related to increasing aridity with increasing distance from the coast.

Seedlings from higher altitudes and also from more inland sources were slow in growth for all traits assessed with high and low altitude provenances often showing the extreme values. For example, seedlings from the lowest altitude sources tended to have lower mean leaf area, height and diameter. The effect of altitude of seed source on growth of seedlings of *Grevillea robusta* was well illustrated by extracting and plotting the first principal component against altitude of seed source (see Figure 5.9). The relationship between this principal component and altitude of seed source was significant (r=0.78). The relationship thus shows the dependence of growth on factors that change with altitude of seed source. As shown in Chapter 5, the first component represented basic growth characteristics in the principal component analysis.

Altitude is a gradient complex involving a number of climatic and environmental variables which may be correlated to a greater or lesser extent. (Austin, 1985). The range of climatic conditions that are influenced by altitudinal change include humidity, evapotranspiration, and temperature. In this study, variation related to altitude of seed source was probably as a result of the general relationship between altitude and temperature. The duration of temperature suitable for growth or photosynthetic activity usually declines with altitude. Lower temperatures and associated shorter growing periods at high altitude sources of *Grevillea robusta* could partly account for the slower growth rates of these sources and may reflect an adaptation to these conditions.

While temperature has a roughly linear relationship with altitude, other climatic and environmental variables can have more complex relationships. Changes in genetically based characters from one population to another may represent either adaptive change or random drift (Hessing, 1989). The almost clearly defined change in most traits along an altitude gradient suggested that adaptive change may be the most important cause of differentiation in the species.

The positive relationship between most growth traits and latitude of seed source was unexpected. The results suggested that seedlings from more southerly sources grew faster than the more northerly sources. This deviated from findings of other experiments in forest trees. In many other studies of northern hemisphere species, negative relationships exist between latitude of seed source and growth traits, such as in *Pinus contorta* (Rehfeldt, 1987) and in Douglas fir (Campbell and Sorensen, 1978).

Temperature and photo period are two critical factors associated with the annual cycles that probably exert strong selection pressures on tree populations (Campbell and Sorensen, 1978). In this study, the range of latitudes involved was small, about three degrees, and there was a possible confounding effect of altitude of seed source on latitude. Because the distribution of the provenances used in this study spans only three degrees of latitude, differences in growth period and day-length between the latitudes may not be significant in influencing growth as much as the effect of altitudinal range of over 900 metres found in the area of natural occurrence of *G. robusta*. The more northerly provenances of the species were those from high altitude. A regression of the two factors on most traits showed that the latitude effect was insignificant in explaining variation in most traits. This could be explained by stronger altitudinal effects that tended to mask the effect of latitude.

As mentioned earlier, there appears to be a general east-west decrease in rainfall in the natural range of *G. robusta* and therefore increase in aridity. Correlations of most seedling growth characteristics with longitude of seed source may be because of selective environmental pressure through increasing aridity. However, the Bunya mountains provenance deviated somewhat from this trend, growing relatively faster for a high altitude source. This may be because of ameliorating effects of the mountainous nature of the source.

Provenance variation and correlations observed between phenotypic traits and environmental and geographic factors of the seed sources could have arisen in two ways. The variation might be attributed to genetic differentiation between provenances, where certain environments have favoured particular genotypes, or to phenotypic plasticity, where environmental conditions evoke particular phenotypes from a common genotype eg. (Jain, 1979). Genetic variation may therefore be important in explaining the performance of a species along environmental gradients. Genetic differentiation within a species in response to habitat differences has been repeatedly observed and has become an established phenomenon (Antovonics, 1976). It is now generally accepted that habitat-correlated genetic variation occurs within widespread species because of natural selection (Stern and Roche, 1974). The range of climatic and environmental conditions in the parent populations of the seedlings of *G. robusta* may be responsible for the variation in their performance. This variation may reflect natural selection in relation to the parent population environment.

7.4 Partitioning of variation among and within provenances

Variance components for individual traits showed that, on average, variability was expressed more among provenances than within. For some traits such as leaf area and height, the percentage of total variance accounted for by individuals in the controlled environment of the glasshouse may be largely masked by the environmental heterogeneity in the field. Heritability estimates obtained in this study should therefore be treated with caution and used only as indicators of the most heritable traits. All heritability estimates therefore should be thought of as figures that give a general idea of the relative strength of inheritance of particular traits (Zobel and Talbert, 1984).

Differentiation between populations also can be studied with regard to the patterns and levels of correlations between different traits. As pointed out in chapter 6, phenotypic correlations at least sometimes may provide accurate approximations of underlying genotypic correlations. An examination of the correlation matrix in Table 6.2 reveals significant intercorrelations between many traits. Correlations between linear growth traits such as height, diameter, leaf area etc. were expected. Correlations such as between internode length and height may be important in selection for height growth. For internode length, both the number of nodes and length of internodes may vary independently among trees, families, and provenances. The possibility therefore exists for recombining them genetically to produce progeny that are in superior height growth. Of importance were the negative correlations as they may indicate whether selection of particular traits will lead to gain reduction in others. In this study leaf length was negatively correlated with most growth traits. Leaf length was also found to have high heritability, suggesting that selection for this trait may lead to decline in genetic gain in other traits.

7.6 Conclusions

From the study of variation in *Grevillea robusta*, the following conclusions were made.

1 There was significant variation between provenances and between families within provenances of *G. robusta* in seed and seedling growth characteristics.

2 Most of the variation was found, on average, to be because of differences between provenances rather than within. This however varied substantially between different traits, with some traits also showing very high proportion of the total variance to be between families.

3 Most seedling growth characteristics exhibited clinal variation associated with geographic locations and climatic factors of the seed source of the parent trees. Altitude showed the strongest effects while the effects of latitude, although somewhat strong, may not have significance because of the small range of latitude of source of the material used in the experiment. Since altitude and temperature of seed source were highly correlated, most traits also showed similar correlations with temperature of seed source. The fastest growing provenances were those from the mid-lower altitude range of the species.

The Bunya mountains provenance (No. 3) was from the highest altitude of seed source used in the study. This provenance deviated substantially from the general altitudinal trends for most growth traits. The provenance may be important in areas where high altitude limits growth of *G. robusta.* (see Chapter 2)

4 The provenances tended to divide into two major groups, based on a combined analysis (PCA) of traits assessed and variables of seed source. Provenances from the coastal areas of New South Wales formed one major group, whereas Bunya Mountains Emu vale and Porters gap formed the other. The Nimbin provenance was separated from the other groups. These groups generally clustered on the basis of the two major sources of the natural occurrence of *G. robusta,* the riverine and the upland dry habitats.

5 Most traits showed high heritability values, which may possibly be related to the generally uniform glasshouse conditions. It was also found that most seed and seedling growth traits were correlated to varying degrees.

SECTION II

CHAPTER 8

VARIATION IN PRODUCTIVITY IN STRESSED AND WELL WATERED SEEDLINGS OF *GREVILLEA ROBUSTA*

8.1 Introduction

The first experiment revealed substantial variation between provenances in glasshouse grown *Grevillea robusta.* The variation closely followed environmental gradients associated with altitude, latitude and longitude (or possibly more correctly, distance from the ocean) of seed origin. Such variation along geographic gradients in many forest tree species has been interpreted in terms of adaptation to the changing environment (Rehfeldt, 1987).

As discussed in Chapter 3, variation found in seedlings of *Grevillea robusta* along geographic gradients may result from selection pressures, in particular temperature and moisture availability on the parent populations. The species occurs in two distinct habitat types; the wet (riverine) and dry upland habitats (see Chapter 2). One of the main differences between the two habitat types is moisture availability and therefore populations of *G. robusta* occurring in the drier habitats may be more tolerant to periods of water deficit than those from the wet habitats as a result of different selection pressures. This may have resulted in differentiation of the populations from these habitats. The second part of this thesis examines this proposition.

According to Grime and Hunt (1975), species and populations of the same species from contrasting habitats may differ vastly in their maximum relative growth rate under optimum conditions. Populations from productive habitats tend to have inherently higher relative growth rates than those from water deficient, nutrient poor or other unfavourable conditions (Grime, 1979). The results of the first experiment suggested that provenances from the wet habitat types generally had higher growth rates than those from dry habitats.

8.2 Mechanisms of drought resistance

Drought stress is a term plant physiologists use to describe both environmental and plant water-deficits (Kramer and Kolowski, 1979). Many definitions that have been coined generally relate drought to climatic indices such as rainfall, temperature, relative humidity and soil moisture. For the purposes of this study, drought will be defined as any period during which seedling and/or soil water deficiencies affect growth and development of seedlings. This may result from either insufficient moisture supply or large seedling demand for water. A drought resistant seedling can be defined as that which suffers relatively little damage under moisture stress conditions, the damage being expressed as yield reduction.

Under natural conditions, drought may result from of a multiplicity of factors or climatic components such as precipitation and its distribution patterns, relative humidity and temperature. In plants, drought stress may be moderated or enhanced by a range of factors such as soil type, topography etc., most of which are difficult to test in the glasshouse.

Plants respond to environmental stress with physiological and morphological adjustments which counter the stress effects and maintain normal functioning. The ability of plants to respond to the stress may result from the environmentally mediated modifications, genetic variation within a species or both (Abrams *et al.*, 1990).

Water is the most limiting factor that may influence a plant's growth. As plants are exposed to internal water stress, many physiological functions are disturbed. In the initial stages of water deficit, only the sensitive processes are altered but prolonged periods of water deficits may lead to gross changes in morphology, anatomy and physiology of the plant. Measurement of morphological traits after a period of stress may provide an insight into the nature of plant adjustment and adaptation to water stress (Jolly *et al.* 1989).

A more general income averaging scheme can be represented as:

$$Y_a = \frac{1}{L} \sum_{l=1}^{L} Y_{1-l}$$

where Y_o is the current year's income.

In the present scheme, $L = 5$ so that:

$$Y_a = \frac{1}{5} \sum_{l=1}^{5} Y_{1-l}$$

[or, equivalently $Y_a = \frac{1}{5} (Y_0 + Y_{-1} + Y_{-2} + Y_{-3} + Y_{-4})$]

Mechanisms of resistance to water stress have been documented extensively in agronomic crops, and to a lesser extent in forest trees. In forest trees, these mechanisms have been described by various authors including Levitt (1972) and Blum *et al.*, (1983) amongst others. Tolerance of trees to water stress is determined by many factors, including a wide range of plant morphological characteristics, osmotic adjustment and stomatal conductance (Seiler and Johnson, 1988). The importance of each varies between and within species. Some morphological mechanisms are associated with modification of shoot characteristics in general and leaf characteristics in particular. For example, small leaves with high specific leaf area (leaf dry weight to leaf area ratio), leaf shedding, heavy cutinisation and pubescence. Other mechanisms may involve whole plant responses such the balance between shoot and root growth (low shoot/root ratio) and also a general reduction in growth.

As mentioned in Chapter 2, *Grevillea robusta* has been observed to possess morphological features that have also been associated with resistance to water deficit in other plants. These include traits such as pubescence on the adaxial surface of the leaves and the stem, wax on the foliage. The presence of such xeromorphic features as deep rooting habit and also the presence of proteoid roots also may increase the efficiency of water absorption and therefore confer resistance to water stress in *G. robusta*. According to Kramer (1983) species capable of avoiding stress by establishing deep root systems or restricting water use under conditions of limiting supply are considered to have the best adaptation to arid and semi arid conditions.

The relative importance of these traits in conferring water stress resistance in *G. robusta* is unknown. Further, knowledge of variation in the species in important traits associated with resistance to water stress may provide an opportunity for selection and genetic improvement for drought resistance. As an important multipurpose tree, *G. robusta* is likely to find increasing use in marginal environments such as semi arid areas where water availability is poor.

Because many trees acclimatise both physiologically and morphologically to water stress, it is of interest to evaluate the existence of such traits in the species and whether there exists variation between the wet and dry upland sources of the species.

8.3 Aims and objectives of the study

The study examined variation in morphological and physiological responses to water stress in glasshouse-grown seedling progeny of five provenances of *Grevillea robusta* from the two habitat types, the riverine and dry upland, compared to well watered seedlings from both habitat types. The Bunya mountains (No. 4) and, Porter's gap (No. 3) are upland provenances located away from rivers and the others, Nimbin (No. 1), Mummulgum (No. 2) and Conondale (No. 5) are riverine sources (Table 8.1; Conondale is designated 12 in Figure 2.1).

The other aim of the study was to determine the extent of interaction between moisture levels and, provenances and families within provenances for different growth characteristics, and to identify the most important morphological characteristics associated with resistance to water stress in *Grevillea robusta.*

8.4 Materials and methods

8.4.1 Seeds

Table 8.1 shows details of the geographic locations and climatic factors of the seed sources used in the study. The seeds were obtained from the Australian Tree Seed Centre, Canberra. Each of the five provenances used was represented by two families.

Seeds from the each family were sown separately in March 1993, following the same procedures as described for the first experiment. A potting mix of equal proportions by volume of peat and vermiculite was used. After emergence of the first true leaves, each seedling was transplanted into eight-centimetre diameter pots containing a mixture of 2:1 pine soil and sand, and then into 12 x 36 cm PVC drainage pots after two months. The seedlings were grown in non limiting soil moisture conditions for three months. Glasshouse bench position effects were reduced by rotating the seedlings in each block every two weeks.

Table 8.1. Geographic locations and climatic parameters of five provenances of *Grevillea robusta* used in the second experiment. (Regions:- NSW = New South Wales; QLD = Queensland). Conondale is designated 12 in Figure 2.1.

Provenance	CSIRO Number	Region	Altitude (m)	Longitude (E)	latitude (S)	Rainfall (mm)	Temp. (°C)
1. Nimbin	17612	NSW	50	153 13'	28 38'	1505	19.7
2. Mummulgum	17617	NSW	100	152 49'	28 50'	1095	19.1
3. Porters gap	17694	QLD	680	151 50'	26 75'	746	17.2
4. Bunya mts.	17633	QLD	1000	151 37'	26 54'	925	15.0
5. Conondale	17956	QLD	150	152 43'	26 44'	1430	19.8

8.4.2 Experimental growth conditions

Glasshouse temperatures ranged from 18 to 25 °C during night and day respectively. As the experiment was carried out during short-day winter conditions, natural day-length was increased to 16 hours using 400 watt/80 m (PAR) metal halide lamps.

8.4.3 Methods

8.4.3.1 Treatments

To study the effects of water stress on *Grevillea robusta,* the seedlings were subjected to water stress treatment and compared with well watered (control) seedlings. Treatments were imposed when seedlings were three months old. Before imposing the stress treatment, all seedlings were drought-hardened by withholding water for three 3-day periods between June 15 and 30, each drying period being terminated by watering to full capacity. At the beginning of the experiment on 5 July 1993, all seedlings were watered to full capacity and excess water allowed to drain for two hours. The bottom of each tube was then sealed with a tape, followed by weighing to the nearest 5 grams. Plastic beads were placed on top of the pots to prevent excessive evaporation. Thereafter, control seedlings were watered daily while the stressed seedlings were watered to the original weight of the pots only when they did not recover overnight from wilting.

8.3.3.2 Experimental Design

The seedlings were arranged on the glasshouse bench in a Randomised Complete Block Design with four blocks. To have seedlings in each block as uniform as possible, seedlings in each family were ranked in order of increasing height and seedlings of similar height allocated to the same block. This minimised the within-block variation and maximised block to block variation.

The seedlings were then randomised separately within each block followed by a random allocation of the two treatments to individual families. Single seedling plots, with one seedling per pot were used. In total, the experiment consisted of five provenances with two families each (5 x 2 = 10 families), two treatments per family replicated four times, giving a total of 80 pots (10 x 2 x 4 = 80 pots). Rotation of blocks (Section 8.4.1) minimised the directional effects such as those of heaters and coolers in the glasshouse.

8.4.4 Assessment

8.4.4.1 Choice of traits

Many traits, both morphological and physiological are used to evaluate resistance to water stress/drought. In this study, seedlings of *G. robusta* were assessed for height, diameter, number of leaves and mean internode length at the beginning of the experiment. Subsequent assessments were carried out every two weeks for traits listed in Table 4.4. Measurement of basic growth traits was done as described for the first experiment (see Chapter 4). In addition, assessments were made of stem colour, pubescence and leaf angle (scores).

8.4.4.2 Harvesting

All seedlings were harvested, dried and weighed at the end of the experiment following the same procedures as described for the first experiment. In addition, the following ratios were also derived: Leaf area ratio, leaf to stem dry weight ratio and percent dry weight. Leaf area measurements were also made using similar procedures as for experiment one. For leaf area measurements, a total of six leaves were sampled from each third of the seedling.

8.4.4.3 Biomass allocation

Biomass allocation patterns in provenances and families within provenances were compared at the end of the experiment. Strauss and Ledig (1985) have suggested that because allocation changes in a specific manner as the plant grows, allocation patterns should be compared by using allometric constants. Since there was only one harvest, it was considered that all seedlings behaved in the same way as they were of uniform age.

Table 8.2: Traits assessed in the second experiment, units used and time of measurement.

Trait	Code	Units	Assessment interval
Height	HT	cm	2 weeks
Diameter	DM	cm	2 weeks
Leaf length	LE_L	cm	2 weeks
No. of Leaves	LE_No	# Count	2 weeks
Pubescence	PB	score	End of experiment
Root dry weight	RT_WT	g	End of experiment
Shoot dry wt	SH_WT	g	End of experiment
Leaf dry weight	LE_WT	g	End of experiment
Stem dry weight	ST_WT	g	End of experiment
Specific leaf wt	SPL_WT	g/cm^2	End of experiment
Root:shoot ratio	RT/SHT	units	End of experiment
Leaf area ratio	LA-R	g/cm^2	End of experiment
Petiole length	PL	mm	end of experiment

8.4.5 Statistical analysis

Analyses of variance were carried out for each trait to determine if there were any significant differences between the water stress and control treatments, between provenances and between families within provenances in response to treatments and also between water stressed seedlings from the two habitat types compared to control seedlings. Diagnostic checks for normality of the data were carried out in the same way as described in the first experiment. In addition, principal component analysis (PCA) was carried out to reduce the dimension of variation in all the traits by combining them into four components for clearer inferences of the patterns of variation. The procedures for PCA analysis are similar to those described in section 4.1.5.3.2.

CHAPTER 9

VARIATION IN SEEDLING GROWTH RESPONSES TO WATER STRESS IN *GREVILLEA ROBUSTA*: RESULTS AND DISCUSSION

9.1 Introduction

This chapter describes the effects of the well watered and stress treatments applied to the five provenances of *Grevillea robusta* derived from the two contrasting habitat types of the natural occurrence of the species; the riverine and the dry upland types

The effects of water stress on various growth traits are discussed, taking into consideration, first, differences in seedling response to water stress compared to well watered seedlings and secondly, differences between provenances and families within provenances in response to treatments. Differential responses to treatments of seedlings from the two habitat types are also discussed. The extent to which water stress affected growth of seedlings, as compared to controls from different sources and habitat types was considered as measure of resistance to water stress.

9.2 Effect of water stress on seedling growth traits.

9.2.1 Observations

Effects of water stress on growth traits of seedlings of *G. robusta* were apparent after the second week. The first signs of water stress included wilting of terminal leaves. Stressed seedlings had reduced rates of growth, leaf expansion, production of new leaves and growth of individual leaves. Water stress did not result in leaf shedding.

An increase in pubescence was also observed in most seedlings subjected to water stress compared to well watered seedlings. Pubescence was mostly concentrated on the terminal shoot and on the apical meristem. Some seedlings showed signs of increased chlorosis and leaf rolling .

9.2.2 The effects of treatments on height growth

After eight weeks, seedlings subjected to water stress were 35 % shorter in height than well watered seedlings, with the well watered seedlings having a mean of 48.38 cm and the stressed seedlings having 31.27 cm (see Table 9.2). Provenances and families within provenances also differed in response to the treatments. The seedlings from the riverine and dry upland sources were also different in height growth in both the well watered and stress treatments.

Analysis of variance showed that the differences between stressed and well watered seedlings were significant at the 1 % level of probability. The differences between provenances and between families within provenances in response to treatments were also significant at the 1 % level of significance (tested as treatments x provenance and family within provenance x treatment interactions respectively; see Table 9.1). Mean height growth of the well watered and stressed seedlings at the end of the experiment for each of the five provenances are presented in Table 9.2.

The mean provenance height growth ranged from 43.64 to 54.26 cm in well watered seedlings and from 26.45 to 34.21 cm in stressed seedlings. The Mummulgum provenance (No. 2) was the tallest and differed significantly (using an LSD test) from all the rest (Table 9.2). The percentage reduction in mean provenance height growth as a result of water stress was highest in Mummulgum provenance, 34.34 %, and highest in the Bunya mountains provenance with 39.7 %. The patterns of height growth of the well watered and stressed seedlings in each of the five provenances are presented in Figure 9.1.

Provenance differences indicate for both well watered and stressed seedlings, the riverine sources were taller than the dry upland sources, with the riverine sources having a mean of 50.43 cm and 33.75 cm for well watered and stressed seedlings while the dry habitat sources had 45.32 and 27.56 cm respectively. (Table 9.2). Analysis of variance (Table 9.1) showed that these differences were highly significant at the 1 % level of probability. The differences between the habitat types were due to lower values in the Porters gap provenance. The Bunya mountains provenance grew almost as tall as the wet habitat type provenances. However, the non-significant interaction between habitat type and treatments (habitat type x treatment interaction) at the 5 % level of probability suggested that seedlings from the two habitat types responded in the same way to the treatments although seedlings from riverine sources were taller than those from dry upland sources in both treatments (see Table 9.2).

Table 9.1. Summary of analysis of variance of height and diameter in well watered and stressed seedlings of five provenances of *Grevillea robusta* from the two habitat types. ** shows significance at the 1 % level of probability and * significance at the 5 % level. The values in parenthesis show the degrees of freedom for the different sources of variation.

Trait	Final height			Final diameter		
Source of variation	MS	F	p-value	MS	F	p-value
Treatment (1)	5571.69	180.29 **	< 0.001	273.85 **	470.79	< 0.001
Habitat (1)	733.34	23.73 **	< 0.001	8.89 **	15.27	< 0.001
Habitat/treatment (1)	0.00	0.00 ns	0.994	5.11 **	8.78	0.005
Treat/prov (8)	82.50	2.67 **	0.015	0.29 ns	0.50 ns	0.852
Treat/prov/fam (10)	74.27	2.40 **	0.019	1.31	2.25 **	0.029
Residual (Error) (48(7))	30.90			0.58		

Table 9.2. The effect of treatments on mean height and diameter growth in five provenances of *Grevillea robusta* from the two habitat types. D and W represent the dry upland and the riverine habitat sources respectively.

(a) Provenance effects for control and stress treatments.

Trait	Final height (cm)		Final diameter (mm)	
Prov I Treatment	Control	Stress	Control	Stress
(1). Nimbin (W)	46.46	28.92	14.26	10.06
(2). Mummulgum (W)	54.26	35.62	14.12	10.22
(5). Conondale (W)	50.56	36.69	14.64	9.75
(3). Porters gap (D)	43.64	26.66	12.75	9.70
(4). Bunya mts. (D)	47.00	28.45	13.10	9.99
Treatment mean	48.38	31.27	13.78	9.95

(b) Habitat effects.

Traits	Final height (cm)		Final diameter (mm)	
Treatment	Control	Stress	Control	Stress
Wet habitat	50.43	33.75	14.34	10.01
Dry habitat	45.32	27.56	12.93	9.85

Insert Figure 9.1 **Effects of water stress on height growth. Not in diskette, enclosed**

9.2.3 The effect of water stress on diameter growth

Well watered seedlings had significantly larger mean diameter growth than stressed seedlings. No significant provenance differences in response to treatments were detected, but families within provenances differed significantly in their response to treatments. Significant differences between seedlings from the wet and dry habitats in response to treatments were also observed.

Analysis of variance showed that the differences between the treatments were highly significant at the 1 % level of probability (Table 9.1). At the end of the eight week stress period, well watered seedlings had a mean diameter growth of 13.78 mm, while stressed seedlings had 9.95 mm (Table 9.2) This represented an average reduction of about 28 % in the stressed compared to the well watered seedlings.

The differences between provenances in response to treatments were not significant but the differences between families within provenances were significant at the 5 % level of probability (see Table 9.1). Porters gap provenance (No. 3) had the lowest mean diameter of 12.75 mm while the Conondale had the highest, 14.64 mm for the well watered seedlings. For seedlings subjected to water stress Porters gap provenance had the lowest diameter of 9.70 mm while Mummulgum provenance had the highest of 10.22 mm in (Table 9.2).

When compared on the basis of the habitat of origin, seedlings from the wet and the dry habitat type differed significantly in diameter growth irrespective of the treatment as indicated by the significant habitat type x treatment interaction at the 1 % level of probability. Diameter growth of seedlings from the dry habitat type was generally less affected by water stress treatments than for seedlings from the wet habitat sources (see Table 9.2). At the end of the experiment, the riverine source seedlings had a mean diameter of 14.34 mm and seedlings from the dry upland sources had a mean of 12.93 mm for well watered seedlings. A similar trend was detected for the stressed seedlings from both habitat types. (see Table 9.2).

It has been observed in other studies that diameter growth is extremely sensitive to environmental stress (Kramer and Kozlowski, 1979). They postulated that cambial growth was generally affected by environmental stress to a larger extent than shoot elongation. As an important component of stem volume, selection for diameter growth is likely to have an important bearing on selection of provenances where the aim of tree planting is to maximise wood production under conditions of moisture deficit.

9.2.4 The effects of water stress on leaf growth characteristics

Water stress significantly inhibited leaf area expansion, rate of production of new leaves and length of leaves in all seedlings by about 48 %, 38 %, and 33 % respectively. Observations showed that these differences were accompanied by an increase in glaucousness and chlorosis although these characteristics were not documented. Although pubescence is a general characteristic of *G. robusta*, seedlings subjected to water stress showed a general increase in pubescence, especially of the apical meristem and the terminal shoots. Chlorosis is a common characteristic of plants under stress and may result from increased production of anthocyanin (Hsiao, 1973). All these traits, ie., pubescence, chlorosis, glaucousness and reduction in leaf growth are associated with water stress resistance in plants. However, leaf area reduction does appear to be the most sensitive indicator of water stress.

9.2.4.1 Leaf area

Highly significant differences in leaf area production were detected between the well watered and stressed seedlings at the 1 % level of probability (see Table 9.3). At the end of the experiment, well watered seedlings had a mean leaf area production of 3278 cm^2 per seedling while seedlings subjected to water stress treatment produced 1722 cm^2, (about 48 % less leaf area) in comparison (Table 9.4).

This study also examined variation in leaf area production between provenances as well as between families within provenances in response to the treatments. Analysis of variance revealed significant ($p < 0.05$) provenance and family within provenance ($p< 0.01$) differences in response to treatments (see Table 9.3). The well watered seedlings had mean provenance leaf area ranging from 2903 cm^2 in the Porters gap provenance (No. 3) to 3507 cm^2 in the Conondale provenance (No. 5) and in stressed seedlings from 1522 to 2010 cm^2 in Porters gap and Conondale provenances respectively (see Figure 9.3).

In both the well watered and the stress treatments, seedlings from the wet habitat type produced more leaf area, on average, compared to seedlings from the dry habitat type. The well watered seedlings from the wet habitat type produced 3399 cm^2 while those from the dry habitat type produced 9 % less leaf area (3097 cm^2) in comparison. The same pattern was observed in stressed seedlings from both habitat types with stressed seedlings from the wet habitat type having a mean of 1884 cm^2 and stressed seedlings from the dry habitat type having 1539 cm^2 (18.3 % less leaf area) (see Figure 9.2). Stressed seedlings from the wet habitat type reduced their leaf area by a higher percentage compared to stressed seedlings from the dry habitat type. However, these differences were not significant at the 5 % probability level.

Table 9.3. Summary of analyses of variance of leaf growth characteristics of well watered and stressed seedlings in five provenances of *Grevillea robusta* from two habitat types. Significance of F-test ** = significant at the 1 % level of probability ($p<0.01$); ** = significant at the 5 % level ($p<0.05$); ns = not significant at the 5% level.

Trait	Leaf area			leaf length			Number of new leaves			Rate of leaf production		
Source of variation	MS	F	p value	MS	F	p value	MS	F	p value	MS	F	p-value
Treatment (1)	5.E+07	863.79**	< 0.001	3094.269	518.64*	< 0.001	342.234	279.86**	< 0.001	13.69	279.86**	< 0.001
Habitat (1)	2617953	45.51**	< 0.001	15.039	2.52 ns	0.119	17.277	14.13**	< 0.001	0.69	14.13**	< 0.001
Habitat/treatment (1)	77590	1.35 ns	0.251	6.706	1.12 ns	0.294	0.002	0.00 ns	0.970	0.0001	0.00 ns	0.970
Treat/prov (8)	129699	2.25*	0.039	17.505	2.93 **	0.009	0.267	0.22 ns	0.986	0.011	0.22 ns	0.986
Treat/prov/fam (10)	198873	3.46**	0.002	19.869	3.33 **	0.002	2.013	1.65 ns	0.120	0.08	1.65 ns	0.120
Residual (Error) (48(7))	57523			5.966			1.223			0.05		

Trait	Leaf dry weight			Percent dry weight			Leaf area ratio			Specific leaf area		
Source of variation	MS	F	p-value	MS	F	p-value	MS	F	p-value	MS	F	p value
Treatment (1)	4330.170	926**	<0.001	25.563	8.14**	0.006	0.754	4.46	0.040	1.82×10^{-5}	0.72 ns	0.471
Habitat (1)	30.486	6.52*	0.014	0.977	0.31 ns	0.579	1.321	7.81	0.007	0.0014	55.34**	< 0.001
Habitat/treatment (1)	1.532	0.33 ns	0.570	0.779	0.25 ns	0.620	0.699	4.13	0.047	0.00034	13.42**	< 0.001
Treat/prov (8)	6.126	1.31 ns	0.261	7.351	2.34**	0.030	0.160	0.95	0.488	0.000029	1.17 ns	0.335
Treat/prov/fam (10)	12.107	2.59*	0.013	3.43	1.09 ns	0.384	0.218	1.29	0.262	0.000035	1.39 ns	0.212
Residual (Error) (48(7))	4.676			3.14			0.169			0.000025		

Traits	Leaf:root dry weight. ratio			Petiole length			Pubescence		
Source of variation	MS	F	p-value	MS	F	p-value	MS	F	p value
Treatment (1)	0.0012	0.05 ns	0.823	43.294	148.8**	< 0.001	4.900	6.94*	0.017
Habitat (1)	0.4018	17.37**	< 0.001	0.0845	0.29 ns	0.592	2.817	3.99 ns	0.062
Habitat/treatment (1)	0.0136	0.59	0.446	0.392	1.35 ns	0.251	0.600	0.85 ns	0.369
Treat/prov (8)	0.012	0.52	0.837	0.558	1.92 ns	0.077	0.073	0.10 ns	0.999
Treat/prov/fam (10)	0.0277	1.20	0.314	0.984	3.38**	0.002	0.900	1.27 ns	0.317
Residual (Error) (48(7))	0.0231						0.7059		

Table 9.4. The effect of water stress on leaf growth characteristics of *Grevillea robusta*. (a) Provenance (Prov) and treatment means and (b) Differences between habitat types. D= Dry and W= riverine habitat (Hab.)

(a) Provenance (prov) and treatment effects

Traits	Leaf area (cm^2)		Leaf length (mm)		Leaf dry weight (g)		No. of New leaves	
Prov Treatment	Control	Stress	Control	Stress	Control	Stress	Control	Stress
1. Nimbin (W)	3412	1801	38.48	23.83	30.64	16.11	11.19	6.54
2. Mummulgum(W)	3279	1722	38.29	24.23	30.63	15.46	10.81	6.70
5. Conondale (W)	3507	2010	39.17	29.06	31.33	17.62	10.25	6.88
3. Porters gap (D)	2903	1522	37.42	25.06	28.50	15.55	11.25	6.80
4. Bunya mts. (D)	3290	1555	37.51	25.83	29.97	15.28	11.56	6.61
Treatment mean	3278	1722	38.17	25.60	30.21	16.00	11.01	6.84

(b) habitat type effects

Traits	Leaf area (cm^2)		Leaf length (mm)		leaf dry weight (g)		No. of New leaves (Number)	
Hab. Treatment	Control	Stress	Control	Stress	Control	Stress	Control	Stress
Wet habitat	3399	1884	38.65	25.70	30.78	16.40	10.25	6.54
Dry habitat	3097	1539	37.46	25.44	29.23	15.42	11.41	7.28

(a) Provenance (prov) and treatment effects

Trait	Rate of leaf production/week		Petiole length (mm)		Leaf Area Ratio		% Dry Weight	
Prov Treatment	Control	Stress	Control	Stress	Control	Stress	Control	Stress
1. Nimbin (W)	2.24	1.36	5.44	3.56	4.73	4.93	42.55	44.58
2. Mummulgum (W	2.16	1.26	5.25	4.11	4.33	4.72	42.60	43.46
5. Conondale (W)	2.05	1.30	5.55	4.28	4.59	5.20	41.29	41.31
3. Porters gap (D)	2.25	1.44	5.63	4.61	4.62	4.49	41.96	42.53
4. Bunya mts. (D)	2.32	1.48	5.65	4.88	4.85	4.66	41.48	43.65
Treatment mean	2.20	1.37	5.50	3.89	4.62	4.80	41.98	43.11

(b). Habitat effects

Traits	Rate of leaf production/week		Petiole length (mm)		Leaf Area Ratio		% Dry Weight	
Treatment	Control	Stress	Control	Stress	Control	Stress	Control	Stress
Wet habitat	2.15	1.31	5.41	3.99	4.60	4.95	42.15	43.12
Dry habitat	2.28	1.46	5.63	3.75	4.73	4.73	41.72	43.09

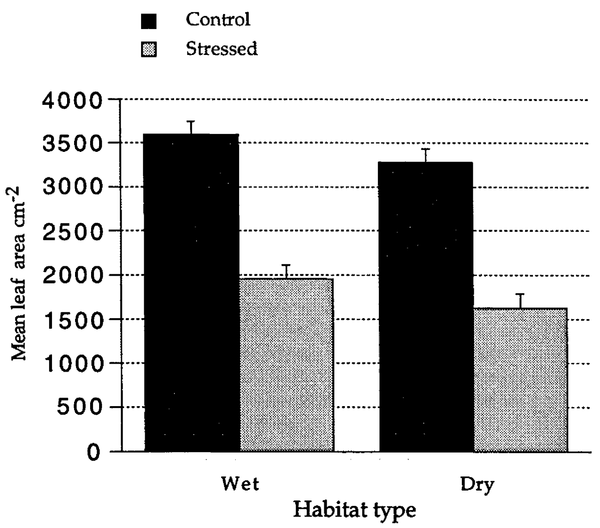
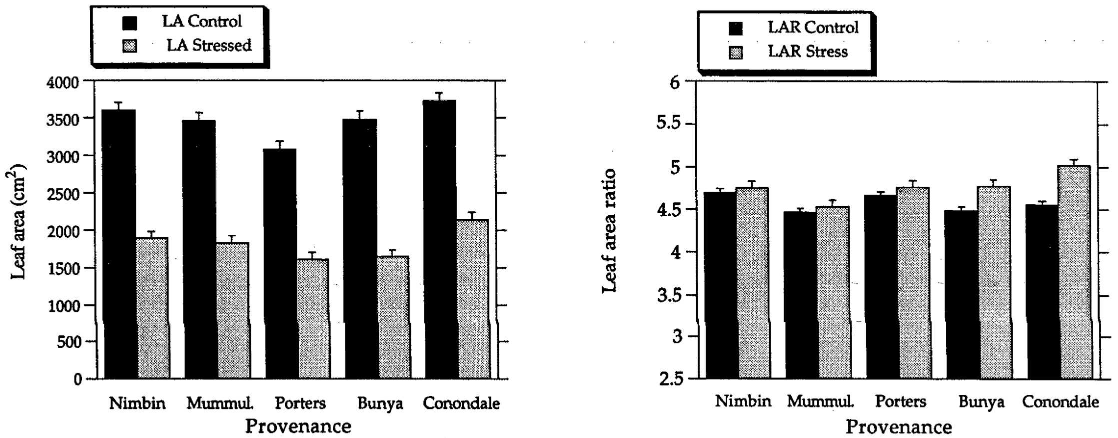
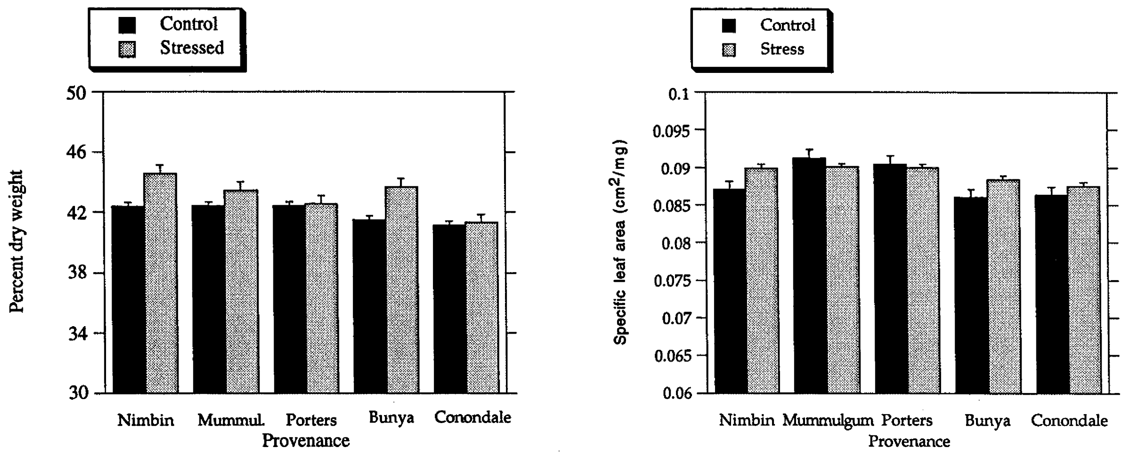


Fig. 9.2. The effect of habitat type on leaf area production for well watered and stressed seedlings.



(a) Leaf area

(b) Leaf area ratio



(c)

Percent dry weight

(d) Specific leaf area

Fig. 9.3. The effect of water stress on leaf growth characteristics in five provenances of *G. robusta*. (a) leaf area, (b) leaf area ratio, (c) percent dry weight and (d). specific leaf area. Vertical bars show the standard error of the mean.

9.2.4.2 Leaf length, number of leaves and rate of production of new leaves

There were statistically significant differences between the two treatments in leaf length, number of leaves and the rate of production of new leaves at the 1 % level of probability ($p < 0.01$) (Table 9.3). Seedlings subjected to water stress had on average, about 33 % shorter leaves compared to the well watered seedlings. Table 9.4 shows the mean leaf length, the number of leaves and rate of production of new leaves at the end of the experiment for each provenance for stressed and well watered seedlings.

Water stress significantly reduced the rate of production of new leaves, with well watered seedlings producing new leaves at a constant rate, an average of five new leaves emerging every two weeks, compared to an average of three new leaves emerging in stressed seedlings. Size of the new leaves produced was smaller in stressed than in control seedlings. In seedlings of *Grevillea robusta,* leaves emerge from the main stem.

Highly significant differences between seedlings from the habitat types were observed at the 1% level of probability in both the number of new leaves and rate of production of new leaves. However, no significant interaction between habitat type and the treatments was detected in leaf length (see Table 9.3). Stressed seedlings from the dry habitat type had on average fewer number of leaves than stressed seedlings from the wet habitat type.

The differences between provenances and families within provenances in leaf length in response to the treatments were significant at the 1 % level of probability. No significant differences were detected between provenances and between families within provenances in response to the treatments both in the number and the rate of production of new leaves (see Table 9.3).

9.2.4.3 Leaf dry weight and per-cent dry weight (% DW)

Well watered seedlings produced an overall mean leaf dry weight of 30.21 g while stressed seedlings had a mean of 16.0 g. These differences were significant at the 1 % level of probability ($p < 0.01$) (Table 9.3). As with leaf area, water stress resulted in a reduction of about 47 % in leaf dry weight in the stressed compared to the well watered seedlings (see Table 9.4).

Provenances did not differ significantly in leaf dry weight in response to treatments at the 5 % level of probability (non-significant provenance x treatment interaction (see Table 9.3). Provenance mean leaf dry weight in the well watered seedlings ranged from 28.50 g in the Porters gap provenance (No. 3) to 31.33 g in the Conondale provenance (No. 5) while for stressed seedlings, the means ranged from 15.28 g in the Bunya mountains provenance (No. 4) to 17.62 g in the Conondale provenance (Table 9.4). Provenance differences in response to treatments were mainly due to the lower values in the Porters gap and Bunya mountains provenances. Significant variation between families within provenance was also detected at the 5 % probability level (see Table 9.3).

Another leaf growth trait examined was the percent dry weight (The ratio of leaf dry to leaf fresh weight ratio). The percentage expressed by this ratio is the percent dry weight (% DW). The ratio gives an approximation of the density of the leaf tissue. This percentage may change independently of leaf thickness. A higher percent dry weight may reflect or can be associated with decreases in leaf area. Analysis of variance showed significant differences between well watered and stressed seedlings at the 1 % level of probability ($p<0.01$). Seedlings subjected to water stress had higher percent dry weight, 43.11 %, compared to the well watered seedlings, with 41.9 %.

Significant provenance differences in response to treatments (provenance x treatment interaction) were detected ($p<0.05$, Table 9.3). Mean provenance percent dry weight in the control seedlings ranged from 41.29 % in Conondale provenance to 42.60 % in the Mummulgum provenance whereas in the stressed seedlings, the mean ranged from 41.31 % in Conondale provenance to 44.58 % in Nimbin provenance (Table 9.4). Separation of means using LSD test showed that provenances in the control treatment did not differ significantly in percent dry weight, but provenances in the stressed treatment did differ significantly. This result suggested that provenances of *G. robusta* responded differently in this trait when subjected to water stress. As mentioned before, a high percent dry weight is associated with a reduction in leaf area.

Significant differences between families within provenances in response to treatments were also detected at the 5 % probability level. However, only the stressed seedlings of families in the Bunya mountains and Conondale provenances differed significantly in percent dry weight from all the rest. No significant differences existed in this trait between seedlings from the wet and dry habitat sources as indicated by the non significant habitat x treatment interaction (see Table 9.3).

9.2.4.4 Leaf area ratio (LAR)

Leaf area ratio is the ratio of the total leaf area to total dry weight per seedling (mm^2/mg). The ratio gives the morphological index of leafiness of a plant and is an indicator of photosynthetic tissue relative to the non-assimilative tissue (Kramer, 1983). It therefore serves as a useful index of the balance between net assimilation and productivity.

The leaf area ratio (LAR) was higher in stressed than in well watered seedlings. These differences were significant at the 5 % level of probability ($p<0.05$). Stressed seedlings had an average ratio of 4.80 mm^2/mg and well watered seedlings an average of 4.62 mm^2/mg. No significant differences between provenances and between families within provenances were detected at the 5 % level of probability (see Table 9.3). Mean provenance leaf area ratio of well watered seedlings ranged from 4.33 mm^2/mg in Mummulgum provenance (No. 2) to 4.85 mm^2/mg in the Bunya mountains provenance (No. 4), whereas the LAR of stressed seedlings ranged from 4.49 mm/mg^2 in Porters gap (No. 3) to 5.20 mm^2/mg in Conondale provenance (No. 5). Seedlings from the riverine and dry upland habitats differed significantly in the LAR at the 5 % level of probability, the ratio being lower in seedlings from the wet habitat type for both stressed and well watered seedlings.

9.2.4.5 Specific leaf area (SLA)

Specific leaf weight (SLA) is the ratio of leaf dry weight per unit leaf area. The ratio of fresh weight to leaf area gives an approximation of leaf thickness. SLA is not an indication of leaf thickness as the ratio between fresh and dry leaf weight may vary. According to Kramer (1983), water stress not only reduces leaf area, but increases leaf thickness thereby increasing dry weight per unit leaf area.

In this study, the treatments did not result in significant differences in SLA. However, seedlings under water stress treatment had slightly more dry weight per unit leaf area, 0.089 compared to the well watered seedlings, which had 0.088 (see Figure 9.3).

The dry upland sources accumulated more dry matter per unit leaf area compared to the riverine sources. Seedlings from both habitat types responded differently in SLA as indicated by the significant habitat type x treatment interaction (Table 9.3). Provenances and families within provenances did not differ significantly in SLA in their responses to water stress.

9.2.4.6 Leaf to root dry weight ratio

Leaf to root dry weight ratio is presented as an index of the balance between the capacities for transpiration and absorption. No significant differences were observed in the ratio between treatments, between provenances and between families within provenances. Differences between seedlings from the riverine and dry upland habitats were significant but the interaction with treatments was non-significant (see Table 9.3). The ratio was higher in stressed than in the well watered seedlings and higher in seedlings from the dry upland than in the riverine sources.

9.3 The effects of water stress on mean internode and petiole length

There were significant differences between the treatments in seedling mean internode length at the 1 % level of probability (see Table 9.5). Well-watered seedlings had a mean internode length of 1.64 cm while stressed seedlings had a mean of 1.24 cm (see Table 9.6).

Results also showed that provenances differed significantly in their response to treatments (significant treatment x provenance interaction) at the 5 % level of significance. However, separation of means using the LSD test showed that provenance differences in this trait were mainly because of the lower values in Porters gap and Bunya mountains provenances.

There were significant differences between seedlings from the wet and those from the dry upland habitat types in mean internode length at the 1 % level of probability but the interaction between habitat types and treatments was not significant at the 5 % level. Seedlings from the wet habitat type had longer internodes than seedlings from the dry habitat type in both well watered and stressed treatments. (see Table 9.6).

Table 9.5. Summary of analysis of variance of mean internode length in well watered and stressed seedlings of five provenances of *Grevillea robusta* from two habitat types.

	Mean internode length		
Source of variation	MS	F	p-value
Treatment (1)	3.181	104.04	< 0.001
Habitat (1)	1.110	36.29	< 0.001
Habitat/treatment (1)	0.011	0.36	0.553
Treat/prov (8)	0.067	2.18	0.044
Treat/prov/fam (10)	0.109	3.56	0.001
Residual (Error) (48(7))	0.031		

Table 9.6. The effect of treatments on mean internode length in five provenances of *Grevillea robusta* from two habitat types: (a) provenance and treatment effects and(b) Habitat effects.

(a) Provenance effects for control and stress treatments

	Internode length (cm)	
Prov I Treatment	Control	Stress
(1). Nimbin (W)	1.57	1.17
(2). Mummulgum (W)	1.79	1.42
(5). Conondale (W)	1.76	1.47
(3). Porters gap (D)	1.55	1.03
(4). Bunya mts. (D)	1.53	1.09
Treatment mean	1.64	1.24

(b) Habitat effects.

	Internode length (cm)	
Hab Treatment	Control	Stress
Wet habitat	1.71	1.35
Dry habitat	1.54	1.06

There were significant differences between treatments in mean petiole length at the 1 % level of probability (Table 9.3). Well watered seedlings had a mean length of 5.50 cm and stressed seedlings 3.89 cm (Table 9.4).

Seedlings from the wet habitat type had slightly shorter petioles compared to the seedlings from the dry habitat for both the well watered and stressed seedlings (see Table 9.4). However, these differences were not significant at the 5 % level of probability. Provenances did not differ significantly in their response to treatments ($p < 0.05$) but families within provenances did differ significantly at the 1 % level of probability (Table 9.3)

9.4 The effects of water stress on biomass production

Table 9.7 shows the analysis of variance of biomass production of different shoot growth components and roots of well watered and control seedlings of five provenances of seedlings of *Grevillea robusta* at the end of the experiment. Highly significant differences existed between treatments in all dry weight traits examined.

9.4.1 Shoot dry weight

There were significant differences between the well watered and stressed seedlings in shoot dry weight at the 1 % level of significance ($p < 0.01$) (see Table 9.7). The average seedling biomass production was about two times higher in the well watered than in stressed seedlings. Well watered seedlings had an overall mean shoot dry weight of 48.03 g and stressed seedlings had 24.60 g (see Table 9.8).

Provenances and families within provenances differed significantly in shoot dry weight production at the 1 % and 5 % levels of probability respectively (provenance x treatment and family-within-provenance x treatment interactions respectively) (see Table 9.7) . This suggested that the effects of treatments were not consistent among provenances and families within them. Mean provenance shoot dry weight in well watered seedlings ranged from 44.53 g in the Porters gap provenance to 50.77 g in the Mummulgum provenance. Table 9.8 shows the effect of treatments on shoot dry weight production in the five provenances of *Grevillea robusta.*

9.4.2 Root dry weight

As with shoot dry weight, significant differences between the treatments existed in root dry weight production at 1 % level of probability (see Table 9.7). Water stress reduced the overall average seedling root dry weight by over 50 %. Well watered seedlings had a mean root dry weight of 27.23 g, while stressed seedlings had a mean of 13.69 g (see Table 9.8).

Significant provenance differences in root dry weight were also observed at the 1 % level of probability. The mean provenance root dry weight in the well watered seedlings was lowest in Porters gap (No. 1), with 25.56 g and highest in Conondale provenance (No. 5) with 30.20 g. In seedlings subjected to water stress, the mean ranged from 12.51 g in Bunya mountains provenance to 14.56 g in Conondale and Nimbin provenances (Table 9.8).

Habitat effects on root dry weight were significant at the 1 % level of significance. The interaction between habitat type and treatment was also significant at the 5 % level of probability. Well watered seedlings from the wet habitat type had a higher mean root dry weight of 29.22 g while well watered seedlings from the dry habitat type had 24.24 g. Similarly, stressed seedlings from the wet habitat type had lower mean root weight than stressed seedlings from the dry habitat type, with 14.45 g and 12.55 g respectively (see Table 9.8)

9.4.3 Stem dry weight

Stem dry weight may be a suitable indicator, from a utilisation point of view of the potential for wood production under different moisture regimes. Highly significant differences between treatments in stem dry weight existed at the 1% level of probability ($p < 0.01$) (see Table 9.7). At the end of the experiment, seedlings subjected to water stress had over 50 % less stem dry weight (6.10 g) than the well watered seedlings which had a mean of 13.69 g per seedling (Table 9.8).

While the effect of habitat type of origin on stem dry weight production was significant at the 5 % probability level ($p<0.05$), habitat x treatment interaction effect was not, suggesting that seedlings from the two habitat types did not differ in their response to treatments. Seedlings from the wet habitat sources had a higher mean stem dry weight than the dry habitat sources in both control and stress treatments (see Table 9.8). Well watered seedlings from the wet habitat sources had a mean stem dry weight production of 14.49 g while those from the dry sources had 12.47. Similar trends existed for stressed seedlings from both sources.

Table 9.7. Summary of analyses of variance of dry weight traits in stressed well watered seedlings of *Grevillea robusta* derived from five sources in two habitat types. The degrees of freedom from each source of variation are shown in parenthesis. (Prov - Provenance, fam - Family). p-value is the probability value. **- significant at the 1 % level of probability and * - at the 5 % level.

Trait	Shoot dry weight			Root dry weight			Stem dry weight			Total biomass		
Source of variation	MS	F	p-value	MS	F	p-value	MS	F	p-value	MS	F	p-value
Treatment (1)	11478.60	848.9**	< 0.001	4139.43	927.2**	< 0.001	1109.239	591.97**	< 0.001	26036.96	2004.6**	< 0.001
Habitat (1)	132.81	9.82**	0.003	257.58	57.69**	< 0.001	12.432	6.63**	0.013	567.94	43.73**	< 0.001
Habitat/treat (1)	6.76	0.50 ns	0.483	26.81	6.01**	0.018	0.024	0.01 ns	0.911	63.60	4.9*	0.032
Treat/prov (8)	14.95	1.11ns	0.375	18.91	4.24**	< 0.001	1.293	0.69 ns	0.698	58.94	4.54**	< 0.001
Treat/prov/fam (10)	24.21	1.79ns	0.086	5.89	1.32 ns	0.246	7.233	3.86**	< 0.001	43.92	3.38**	0.002
Residual (Error) (55)	13.52			4.46			1.874			12.99		

Trait	Root:shoot ratio		
Source of variation	MS	F	p-value
Treatment (1)	0.000154	0.02 ns	0.891
Habitat (1)	0.052146	6.41*	0.014
Habitat/treatment (1)	0.003086	0.38	0.541
Treatment/prov (8)	0.003281	0.40	0.914
Treatmen/prov/fam (10)	0.009773	1.20	0.311
Residual (Error) (55)	0.008139		

9.4.4 Total biomass production

There were strongly significant differences between the treatments in seedling total biomass production at the 1 % probability level ($p < 0.01$) (Table 9.7). Water stress reduced seedling total biomass production by an overall average of about 50 %. The mean seedling total biomass was 71.29 g for the well watered and 35.90 g for the stressed seedlings (Table 9.8).

Table 9.8 Mean dry weight traits in well watered and stressed seedlings of five provenances *Grevillea robusta* from the riverine and dry habitat types.

(a) Provenance effects for control and stress treatments

Trait	Shoot dry weight		Root dry weight		Stem dry weight		Total biomass		Root:Shoot ratio	
Prov | Treatment	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress
(1). Nimbin (W)	49.48	25.07	28.00	14.56	13.86	5.93	72.45	36.55	0.62	0.66
(2)Mummulgum (W	50.77	25.07	29.46	14.24	14.72	6.81	75.36	36.70	0.65	0.67
(5). Conondale (W)	48.28	26.10	30.20	14.56	14.91	6.49	76.44	38.80	0.57	0.62
(3). Porters gap (D)	44.53	23.26	22.56	12.60	11.83	5.72	63.21	34.02	0.60	0.59
(4). Bunya mts. (D)	47.08	23.49	25.92	12.51	13.11	5.55	69.00	33.43	0.71	0.65
Treatment mean	48.03	24.60	27.23	13.69	13.69	6.10	71.29	35.90	0.63	0.64

(b) Habitat effects for control and stress treatments.

Trait	Shoot dry weight		Root dry weight		Stem dry weight		Total biomass		Root:Shoot ratio	
Hab Treatment	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress
Wet habitat	49.51	25.41	29.22	14.45	14.49	6.41	74.75	37.35	0.66	0.66
Dry habitat	45.81	23.38	24.24	12.55	12.47	5.64	66.11	33.73	0.58	0.61

Provenances differed significantly in seedling total biomass production in response to the treatments at the 1 % level of probability (see Table 9.7). The mean provenance seedling biomass production ranged from 63.21 g in the Porters gap provenance (No. 3) to 76.44 g in the Conondale provenance (No. 5) for the well watered seedlings and from 34.02 g in Porters gap provenance (No. 1) to 38.80 g in Conondale provenance (No. 5) for seedlings subjected to water stress (Table 9.8). Highly significant differences between families within provenances in seedling total biomass production in response to treatments were also detected at the 1 % level of probability (see Table 9.7).

Seedlings from the wet habitat sources had significantly higher seedling total biomass production than those from the dry habitats at the 5 % level of probability. On average, seedlings from the dry habitat type had a lower percentage reduction in seedling total biomass in response to water stress than seedlings from the wet habitat type.

9.5 Effects of water stress on biomass allocation: Root-shoot dry weight ratio

The root to shoot dry weight ratio was slightly higher in the stressed (0.64) compared to the well watered seedlings (0.63) and in seedlings from the wet upland habitat type compared to the riverine sources. The ratio ranged from 0.57 in Porters gap provenance to 0.71 in Conondale provenance for well watered seedlings and from 0.59 in Bunya mountains to 0.67 in mummulgum provenance. No significant differences were detected between the treatments and between the provenances and families within provenances in the ratio (Table 9.7)

9.6 Pubescence

Grevillea robusta generally shows a high degree of pubescence, mostly on the lower side of the leaves and on the upper stem. In this study stressed seedlings were found to have a general increase in pubescence towards the tip of the seedlings. The scores for this trait were therefore based on scoring for the trait on the terminal quarter of each seedling.

There were significant differences between well watered and stressed seedlings. Stressed seedlings showed higher degree of pubescence with a mean score of 2.60 compared to the well watered seedlings, which had a mean score of 3.30. Seedlings from the two habitat types also differed significantly ($p<0.05$) in the degree of pubescence. The dry upland sources were more pubescent, with a mean score of 2.62 whilst the riverine sources had a mean of 3.17. However, the interaction between habitat type effect and treatments was not significant (see

Table 9.3). No significant interactions were found between the treatments and provenances and families within provenances.

9.7 Patterns of variation in water stress resistance: Principal Component Analysis

To study the overall pattern of variation in water stress resistance between seedlings of the five provenances and between the two habitat types, all traits assessed in the experiment were subjected to a Principal Component Analysis. The main purpose of using PCA analysis in this study was to compare seedlings from the five different sources and two habitat types subjected to the well watered and stress treatments on the basis of all traits considered together. Also included were geographic locations and environmental parameters of seed source, with no distinction being made between them and the traits in the analysis. Principal Component Analysis combined all the variables into a reduced set of five linear principal components, with each component containing loadings for each variable included. The relative loadings of the variables on each principal component can be used to determine variables that contribute most to the separation of the different groups. The components were subjected to varimax rotation in order to maximise the variance in each.

The results showed that the first four components together accounted for 91.38 % of the total variation of the original data and therefore negligible information was lost. The percentage of variance accounted for by each of the four components and the loadings for the different variables are given in Table 9.9. The numerical importance of loadings in a PCA does not necessarily translate into biological importance and therefore the interpretation must be subjective and should depend on the purpose of the analysis (Campbell, 1979).

The first principal component (axis) accounted for 59.37 % of the original variation and possessed largest factor score coefficients for growth characteristics particularly leaf growth traits. This component separated the provenances on the basis of seedling growth characteristics and was interpreted as representing general plant size as determined by the number and size of leaves. This component also showed the importance and sensitivity to water stress of leaf characteristics in seedlings of *G. robusta.*

The second principal component accounted for 16.86 % of the total variance and had heavy loadings on geographical and environmental parameters of altitude, temperature and rainfall. This component was interpreted to represent factors of the of seed source environment. The third and the fourth components accounted for 10.47 and 4.68 % of the total variance respectively. The third component provided a contrast between latitude and longitude of seed source and various ratios, ie., specific leaf area, and leaf to root dry weight and percent dry weight. This may indicate the adaptive value of these ratios in the habitat types, which occur across a longitudinal gradient (Increasing aridity with increasing distance form the coast (see Harwood, 1992)).

The plot of the provenances on the first two components is given in Figure 9.4. The plot showed a clear separation of provenances based on their habitat types and the treatments. Provenances from the same habitat type (riverine or dry upland) were clustered together.

Table 9.9. Principal component analysis and factor loadings, latent roots, percent accounted for and cumulative percentage of the first four principal components (PC-1 to PC-4) in seedling growth traits measured in five provenances of *G. robusta* from two habitat types. The first five traits having the heaviest loadings in each component are underlined.

	Principal Components			
Traits	PC-1	PC-2	PC-3	PC-4
Final Height	0.2515	- 0.0276	- 0.0410	- 0.0300
Diameter	0.2421	0.0935	0.0134	- 0.0892
Leaf area	0.2570	0.0760	- 0.0192	0.0654
Leaf length	0.2598	0.0126	- 0.0301	0.0384
No. of new leaves	0.2728	- 0.0985	0.0663	0.0159
Leaf dry weight	0.2658	- 0.0118	- 0.0033	0.0260
% dry weight	- 0.1185	0.0136	0.3525	- 0.0754
Leaf area ratio	- 0.0448	0.0389	- 0.1182	0.7532
Specific leaf area	- 0.0187	0.2743	- 0.4060	- 0.3916
Leaf to root wt. ratio	0.0238	- 0.0649	0.5576	- 0.0533
Internode length	0.2093	0.1846	- 0.0357	0.0436
Petiole length	0.2515	- 0.0195	- 0.0205	- 0.0124
Shoot dry weight	0.2660	0.0359	0.0439	0.0040
Root dry weight	0.2518	- 0.0010	- 0.0617	- 0.0137
Stem dry weight	0.2487	0.0772	- 0.0025	- 0.0587
Total seedling biomass	0.2527	0.0720	- 0.0013	- 0.0294
Shoot:root ratio	- 0.0522	0.4438	- 0.2134	- 0.1546
Altitude	0.0045	- 0.4507	- 0.1699	- 0.0397
Longitude	- 0.0140	0.1949	0.2903	- 0.2339
Latitude	0.0455	0.2108	0.4392	0.0645
Temperature	- 0.0145	0.4578	0.1064	0.0315
Rainfall	0.0094	0.3703	- 0.0414	0.4034
Latent roots	**14.843**	**4.214**	**2.617**	**1.169**
Percentage accounted for	**59.37**	**16.86**	**10.47**	**4.68**
Cumulative %	**59.37**	**76.23**	**86.70**	**91.38**

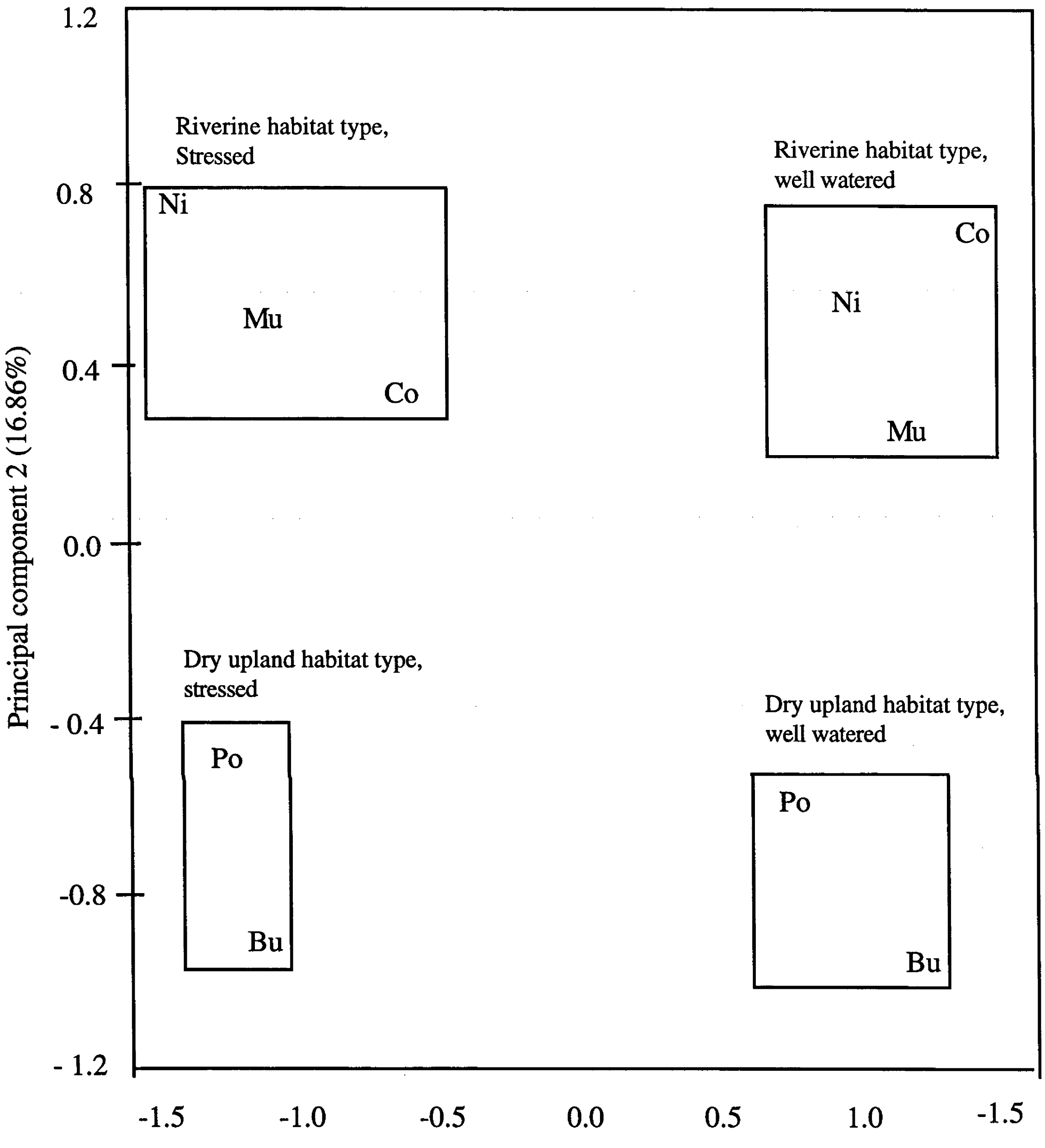


Fig. 9.4. The relationship between the first and the second principal components for traits assessed in five provenances derived from the riverine and dry upland habitat types (Ni - Nimbin, Mu - Mummulgum, Po - Porters gap, Bu - Bunya mountains, and Co - Conondale).

CHAPTER 10

GENERAL DISCUSSIONS AND CONCLUSIONS ON STUDY OF GROWTH AND PRODUCTIVITY OF WELL WATERED AND STRESSED SEEDLINGS OF *GREVILLEA ROBUSTA*

10.1 Introduction

Gross reductions in growth of seedlings of *Grevillea robusta* were observed, regardless of the seed source or habitat of origin, when the seedlings were subjected to water stress. These results conform with the general observation that water deficits generally reduce plant size through reduction in cell division, enlargement and differentiation (Kramer, 1983). In this study, water stress treatment significantly reduced height and diameter growth by 36 % and 27 % respectively whereas leaf area, number of leaves, total seedling biomass and root biomass were reduced by 48 %, 51 %, 50 % and 49 % respectively. Reductions of this magnitude were expected, given the high degree of sensitivity of growth to water stress (Hsiao, 1973).

Seedling progeny from the riverine and the dry habitat types differed significantly in growth for most of the traits assessed in both the control and water stress treatments. The more inland dry upland sources were characterised by slower growth compared to the coastal riverine sources irrespective of the treatment. However, Bunya mountains provenance differed a little from the other dry upland source, possibly as a result of ameliorating effects of aspect and the mountainous nature of its natural occurrence. The study also showed significant provenance and families within provenance variation in response to water stress.

10.2 The effects of water stress on growth of seedlings

During the eight weeks of treatment, growth in most traits such as leaf area, number of leaves, shoot and root dry weight and seedling total biomass of well watered seedlings almost doubled, whereas growth of stressed seedlings only increased slightly. For all traits assessed, differences between well watered and stressed seedlings were highly significant (Table 9.2). On average, seedlings from parent trees in dry upland sites experienced less reduction in growth compared to the riverine sources when subjected to water stress. The riverine and the dry habitat sources also differed significantly in growth traits such as height, diameter and leaf area across both treatments while in some traits such as leaf length, these sources did not respond differently to treatments. Results of principal component analysis suggested that different selection pressures, probably because of differences in moisture availability between the two habitat types may have produced the differences in response to water stress.

Although the responses of provenances may have been due to water stress, differences in most traits seemed inherent and most likely because of selection in the natural environment. Porters gap, a dry upland source had inherently slower growth, a characteristic observed in most drought resistant populations (eg. Larson and Mathes-Sears, 1991).

10.3 Morphological mechanisms for coping with water stress in and variation between habitat types, provenances and families within *Grevillea robusta* provenances

Plants often occur under conditions unfavourable to growth, and may be commonly under water stress in their natural environment. Identifying the mechanisms of drought resistance between populations is often difficult (Potvin and Warner 1983). In some cases adaptive responses appear to be morphological while other mechanisms may also be physiological. Morphological attributes are predominantly associated with differences in the capacity for dehydration avoidance, with root and leaf adaptations playing an especially important role in promoting better plant water balance. Physiological mechanisms include osmotic adjustment, stomatal regulation and relative growth of leaves, stem and roots (Kramer, 1983; Blum *et al.*, 1983).

In this study, water stress resulted in a number of morphological responses in seedlings of *Grevillea robusta*, which included an overall reduction in shoot and root growth. Among the shoot growth components, leaf growth was the most affected, which in turn tended to reduce stem and root growth and consequently total biomass.

Leaf area is responsible for both loss of water by evapo-transpiration and for carbon fixation by photosynthesis. It therefore forms a critical link between productivity and water or drought stress. Under conditions of water deficiency, leaf growth is one of the first physiological processes to be affected (Kramer, 1983). Physiological components known to determine leaf area include the rate and duration of individual leaf growth, and the rate of leaf production. A reduction in leaf area is an important mechanism for controlling plant water loss through respiration and yield under stress and genotypes with smaller leaves have an advantage under water such conditions (Blum, 1988). In this study, water stress significantly reduced the number of leaves produced by seedlings of *Grevillea robusta* but the most important response associated with leaf growth was the reduction in leaf area. This trait showed substantial reduction (48 %) in stressed compared to well watered seedlings. The importance of leaf growth characteristics in coping with water stress was emphasised by their heavy loadings in the principal component analysis (Table 9.4). The effect of leaf area is probably the most important means by which water stress influences productivity as has been observed in other species (eg., Metcalfe *et al.*, 1989).

Various ratios associated with leaf growth were also derived from basic measurements. These included specific leaf area, leaf area ratio and percent dry weight. Specific leaf area (SLA) (the ratio of leaf dry weight per unit leaf area) was higher in stressed compared to well watered seedlings although the differences were not significant. Seedlings from the wet and the dry habitat types differed in SLA, with the dry upland sources having higher SLA, suggesting thicker and more dense leaf. This may reflect inherent differences in leaf anatomy between the sources.

Water stress imposed during periods of leaf development has been shown to cause changes in cell size and in the number of mesophyll cells. These alterations of the internal leaf anatomy change CO_2 and water vapour exchange in a way that results in higher water use efficiency (Nobel, 1980). According to Kramer (1983), water stress not only reduces leaf area but also increases leaf thickness thereby increasing dry weight per unit area. In a study of a comparison of leaf area growth between Eucalyptus species (Mooney *et al.*, 1978), which showed that leaf area of species from the driest regions averaged about one third of those from the wettest habitats and their specific leaf weight about three times higher.

Percent dry weight also gives an approximation of the density and higher values may be associated with decreases in leaf area whereas the leaf area ratio serves as a morphological index of leafiness. In this study, stressed seedlings had more dense leaves and more leaf area per total seedling dry weight compared to well watered seedlings.

There are other mechanisms that allow plants to cope with water stress without irreversibly affecting leaf area such as changes in leaf angle (Turner and Begg, 1991). These changes reduce the radiation load on leaves and allow the plant to dissipate less heat as latent energy and may result from differential turgor in the individual cells, leading to a rolling of the lamina. This reduces transpiration and enables leaves to survive longer under drought conditions (O'Toole and Cruz, 1979). In this study, seedlings of *G. robusta* subjected to water stress showed a marked degree of leaf rolling although no consistent variation was observed between the habitat types or provenances in this characteristic.

Water stress has been found to greatly increase deciduousness in some semi deciduous forest tree species, eg., Black walnut (Carpenter and Hannover, 1974). Although *G. robusta* experiences deciduousness in its natural environment, the species did not shed its leaves when subjected to water stress.

Pubescence is an important characteristic of plants in moisture deficient conditions. It has been repeatedly observed that pubescence increases with increasing aridity within the same species growing in different areas and also increases during dry seasons (Johnson, 1975). Plants that grow in arid habitats tend to be more pubescent than plants of the same species growing in more mesic habitats. The mechanisms by which pubescence is associated with water relations in plants is still obscure but it is thought to increase reflection of radiation by the leaf thereby decreasing conductance of water through the boundary layer of the leaf (Johnson, 1975). The role of leaf pubescence in *G. robusta* is unclear, but it clearly increased as a result of water stress.

Significant provenance variation in response to treatments was observed in leaf area, leaf length and percent dry weight but not in leaf area ratio, number of leaves, rate of leaf production and leaf pubescence. Families within provenances also differed in the same leaf traits as for provenances in addition to leaf dry weight.

In summary, most leaf growth characteristics varied in a consistent manner when seedlings from the riverine and dry upland habitats were compared. Seedlings from the dry upland sources tended to have smaller and thicker leaves, with high specific leaf area.

From the perspective of adaptation to moisture stress, especially under field conditions, *Grevillea robusta* appears to have other potential adaptive advantages. The species has been reported as having a deep rooting habit (Harwood, 1992) and variation in root growth characteristics between some families has been observed under field conditions. Some send most of their roots vertically down whilst others spread their roots horizontally (Harwood, Pers. comm., 1993). The implication is that the deep rooting trees may be more drought resistant that the shallow rooted ones.

In this study, length of roots could not be measured because the roots reached the bottom of the pots within the first three weeks. However, some provenances appeared to have a conservative strategy of more roots per unit leaf area supported (Leaf to root dry weight ratio) when subjected to water stress (see Section 9.2.4.7). This would allow greater development of dehydration tolerance during periods of water deficit. No significant provenance variation was detected in this trait.

There exists a relationship between root development and the amount of water absorbed from the soil. A highly developed root system is a mechanism by which plants can cope with water deficits through an increase in the efficiency of absorption and relative resistance to water deficit (Quisenberry, 1982). Development of extensive root systems, unlike many other mechanisms of drought resistance can only occur through use of photosynthate produced by the plant with a corresponding reduction in other growth components (Fischer and Turner, 1978). This is more pronounced at the early stages of tree development.

The relative allocation of biomass between roots and shoots is another means by which plant flexibility can mediate environmental stress. The shoot to root ratio can represent the balance between water absorption and loss through respiration and evaporation. High shoot to root ratios have been shown to favour rapid growth of plants in moist conditions (Ledig, 1976), whilst lower ratios optimise growth under conditions of moisture stress through allocation of more biomass to roots (eg., Fischer and Turner, 1978). According to Hsiao (1973), alteration of shoot to root ratio balance in seedlings may improve the regulation of plant water relations, resulting in avoidance of low xylem water potential that can cause stress in young seedlings. Larger roots systems can maximise water uptake and smaller shoots minimise transpirational water loss.

The effect of water stress on the relative allocation of growth between leaves and roots of G. *robusta* showed that water stress resulted in more photosynthate being allocated to roots than leaves. However, no significant preferential allocation of photosynthate to roots was observed in stressed compared to the well watered seedlings as has been documented in other plants under similar conditions (eg., Rhoads and Palardy, 1993). No differences were found between provenances and between families within provenances. However, when compared on the basis of the habitat type, the ratio was significantly higher in stressed seedlings from dry upland habitats compared to stressed seedlings from the wet riverine habitat type. As noted earlier, *G. robusta* has a very deep rooting habit but at the same time its root growth is severely restricted by root checking. It is likely that nature of the experiment did not allow the expression of root growth as most seedlings had hit the bottom of the pots at the third week of the experiment.

There exists an inverse relationship between growth rate and stress resistance (Grime, 1979). A slow rate of growth in plants has been identified as a drought resistance trait. There are several direct and indirect ways in which slow rates of growth confer water stress resistance; by minimising the growth respiration associated with production of new tissues, and indirectly by reducing carbon demands for growth (Chapin III *et al.*, 1993). In this study, with the possible exception of the Bunya mountains provenance, the more inland dry upland habitats sources exhibited slow growth in all traits assessed compared to the coastal wet habitat sources in both the control and stressed seedlings. Similar differences have been observed in Loblolly pine (*Pinus taeda*) under controlled growth conditions with seedlings from the driest climates being more tolerant to water stress than those from moist environments (Bongarten and Teskey, 1987).

From the results of this study, it is evident that inland sources *G. robusta* from the dry upland sites, in particular the Porters gap provenance may possess a stress-avoidance strategy characterised by slow growth of seedlings, and reduction in leaf size.

10.4 The effect of habitat of origin

Differentiation in plant populations in response to habitat variation has been well documented and considerable information exists on differentiation and adaptation in response to soil moisture differences (eg., Pharis and Ferrell, 1966). Seedlings of *Grevillea robusta* from the riverine and dry upland habitats differed in most traits assessed in both the well watered and the stressed treatments. The dry upland sources were on average characterised by slower growth rates, smaller leaf area and higher shoot to root dry weight ratios.

The riverine sources of *Grevillea robusta* may have better access to soil moisture than suggested in the precipitation data. Apart from the seasonal rainfall, these sources may access underground water easily because of the deep rooting habit of the species and its proximity to rivers. In contrast, the dry habitat sources are located on upper dry slopes away from gullies and streams and are therefore exposed to soil moisture depletion. Similar studies have been done in other tree species to try and explain differences in water relations in terms of habitat of origin of populations. In some cases, variation in water stress resistance has been found to have a relationship with the habitat type from which the plant originated. For example, Carpenter and Smith (1975) found differences in stomatal frequencies and found that trees inhabiting dry sites had more stomata per unit area than those inhabiting more mesic sites, a characteristic that confered water stress resistance in the trees. Similarly, one might expect moisture stress conditions to exert strong selection pressures in dry upland sources of *G. robusta*.

According to Glover (1990), it is common for genotypes from drier habitats to have inherently slower rates of growth. Similarly, the differences observed in *G. robusta* may reflect adaptations to different moisture regimes in the natural occurrences of the parent trees in the wet and dry upland habitat types.

10.5 Patterns of variation in response to water stress

Results of the principal component analysis showed that well watered and stressed seedlings of the riverine and dry upland habitats can be separated into four discrete groups on the basis of linear combination of all traits and some factors of the seed source environment. These groups represent provenance-combinations from both habitat types. Provenances from the same habitat type and treatment group were similar to each other and different from other treatment-provenance groups.

As mentioned earlier, natural selection, possibly as a result of differences in moisture availability in the habitat types may have produced these differences. The results of the principal component analysis supported the concept that fitness or adaptation to a particular environment depends on a coordinated set of traits which act together to produce a given response (eg., Chapin III *et al.*, 1993). Distinct inherent differences were apparent, both between provenances and between seedlings from the two habitat types. However, interactions among major stress factors may be important in determining the performance of a species and its distribution. While water deficiency is an important stress in plants, its effects can be modified by other factors such as soil type, and other environmental factors as temperature and wind.

10.6 Conclusions

1 There were significant differences between the well watered and stressed seedlings of *Grevillea robusta* in all traits assessed. Gross reductions of up to 50 % in several growth traits showed that the species is sensitive to water deficit. Both root and shoot growth components were affected by water stress, although shoot growth and in particular leaf area was the most important response to water deficit. *Grevillea robusta* copes with water stress by dehydration avoidance through reduction in leaf area which in turn reduces growth. Avoidance determines a decrease of transpiration level or an increase in

extracting water from the soil. Traits related to dehydration avoidance include leaf and root traits.

2 There was significant variation between provenances and between families within provenances in response to the treatments. Despite the small number of families per provenance used, the family component of variation appeared, for most traits assessed, to be more important than the provenance component. *Grevillea robusta* most common response to water stress was a substantial reduction in leaf area. Although water stress significantly reduced the shoot to root ratio, there were no significant differences between provenances.

3 Principal-component analysis also confirmed, as shown by the high loadings on leaf area in the principal component axis that *G. robusta* copes with water stress by severely restricting leaf area expansion and production of new leaves.

4 The study showed that dry upland sources of *G. robusta* may be better adapted to moisture deficit than the riverine sources as a result of natural selection in the natural environment. However, as suggested by results of growth of the Bunya mountains provenance, other factors such as aspect and the ameliorating effect of altitude may modify the effects of moisture deficiency under local conditions.

This study compared morphological responses to water stress of seedlings of *Grevillea robusta* from sources characterised by variation in moisture availability. The physiological basis of the provenance and family differences in seedling response to water stress was not investigated directly but the morphological responses observed may have a physiological basis.

CHAPTER 11

GENERAL DISCUSSION AND CONCLUSION TO THE STUDY OF *GREVILLEA ROBUSTA*

The studies reported in this thesis examined first, variation between and within provenances of *Grevillea robusta* in growth characteristics and secondly, variation between five provenances derived from the riverine and dry upland sources in productivity and growth under conditions of water deficit. The need for a study of *G. robusta* was identified as due to the increasing importance of the species in agroforestry and rural afforestation in many countries and the fact that no comprehensive studies have been conducted in the species. With the increasing planting of the species in more arid and semi areas, identification and quantification of variation in resistance to water stress may be important. Selection and breeding for drought resistance is likely to play an important role in achieving better performance under these conditions.

The results of the study of variation in growth in *Grevillea robusta* (Chapter 5) showed significant provenance variation with a somewhat smaller magnitude of differences between families within provenances. This variation in most traits was distributed between provenances. The variation found in the species was consistent with the expectations of a species occurring in a high environmental heterogeneity. The highly heterogeneous conditions of the natural occurrence of *G. robusta* may have led to substantial differentiation within the species.

Provenance variation in *G. robusta* also followed clinal patterns associated with geographic location and climatic parameters of seed source. Altitude, longitude, and temperature of seed source showed the strongest effects on growth of seedlings. Altitude (or more correctly, environmental factors that change gradually with altitude) was the most important factor in differentiation. These patterns varied widely amongst traits. For example, height and diameter growth showed strong clinal performance while leaf length did not.

Results of principal component analysis showed that the lowland coastal provenances were grouped together on the basis of all traits assessed together. Provenances from higher altitudes, ie., Bunya mountains, Porters gap and Emu vale were more divergent while the rest were more clustered together. However, the Bunya mountains source grew somewhat faster than would be expected for this altitude, compared to other lower altitude sources. These results suggested the presence of ecotypes evolved due to local environmental selection pressures.

Phenotypic correlations between different traits showed that most were significantly but not strongly correlated (Chapter 6). For example, height and diameter growth and shoot and root dry weight traits were significantly correlated with leaf area. Few negative correlations were found but most were associated with ratios. Leaf length was the only basic trait negatively correlated with other traits.

Most traits assessed in seedlings of *G. robusta* showed widely differing heritability values. The results, presented in Chapter 6, showed that some traits, eg., height and diameter growth are heritable and it will be possible to improve them through breeding. The existence of substantial provenance and family within provenance components of variation suggested that it is possible to carry out this selection at both levels of variation and to obtain gains in an improvement programme.

Results of the study comparing productivity in well watered and stressed seedlings of *Grevillea robusta* are presented in Chapter nine. Water stress resulted in large reductions in growth. The magnitude of reduction varied significantly among traits. However, reduction in leaf area was identified as one of the most important mechanisms of coping with water stress. Other associated leaf traits such as pubescence and leaf rolling appeared to be important as well. Various ratios such as specific leaf area, leaf area ratio and leaf to root ratio also reflected changes in leaf size and shape and the balance between the evaporative surface and other growth components.

Variation between seedlings from the riverine and dry upland habitat types in response to treatments was also noted in some traits. The differences between these sources were mainly due to differences in response of the Porters gap provenance.

The results suggest that moving seed from riverine sources to drier conditions may lead to potential failure or slow growth. The riverine sources of *Grevillea robusta* may be expected to exhibit rapid growth under favourable conditions, but are likely to be characterised by poor drought adaptation. Conversely, moving sources from drier upland sites to areas with high rainfall may result in unacceptably slow growth, despite the site conditions, due to the inherently slow growth. One exception to this were the two families of the Bunya mountains provenance. This may be due to micro-environmental factors that could not be identified in the experiment. The results of experiment one revealed that the same families deviated mostly from the trends with altitude and temperature of seed source. The mountainous nature of the seed sources could play an important role in the observed characteristics.

The possibility for selection of sources resistant to water stress in *G. robusta* exists. Despite the small number of families used in each provenance, substantial variation between families within provenances in response to water stress existed. This may allow for selection of trees that are resistant to water stress and at the same time capable of rapid growth. Of significance were the treatment interactions at the family-within provenance level. These interactions suggested that some families within a provenance were more responsive to water stress than others. The large between families within provenance variation may allow for selection of families that are resistant to conditions of water deficit.

Yield reduction is one of the consequences of selecting drought resistance genotypes. Hoffman and Parsons (1991) have suggested a stepwise rather than a concurrent selection ie., selecting for drought resistance first, followed by a selection for optimal yield under non-limiting conditions among the selected genotypes. This is because the heritability of yield is reduced under stress conditions. Therefore selection for yield under stress conditions is inefficient as the variation under stress is likely to be environmental than genetical. Conversely, Jolly *et al.*, (1989) has suggested that under such conditions, it is desirable to select for fast growth, followed by selection for drought resistance.

Breeding of multipurpose trees may require a departure from the traditional criteria of using yield as selection criterion. A tree's yield results from a complex interaction of physiological and morphological processes and while it can be used effectively in provenance evaluation in non stress environments, it cannot be used as an effective and absolute measure of adaptability for stress environments. As superior performance under stress environments is related to the 'residual' effect of high yield, it is common for genotypes with high stress tolerance to have a somewhat low mean yield in non-stress environments (Glover, 1990). It is therefore desirable to select for fast growth and then for resistance to water stress

From the study of *Grevillea robusta* the following conclusions are drawn: That *Grevillea robusta* is highly variable in growth characteristics and also in productivity under water stress conditions. Since variation observed in these studies may reflect variation of the species in its natural environment, it is possible to exploit this variation in a breeding programme. It may also be possible to carry out a gradual genetic enrichment of the land races of *G. robusta* occurring in many tropical and subtropical countries, where the species is most important.

Much of the variation in the species is found between provenances. It is possible to exploit these provenances for planting in different geographical areas and in different environmental conditions.

The substantial variation found within the species in productivity under water stress conditions may be associated with habitat of origin. Planting of the species in different climatic conditions may exploit this variation through matching of habitat type of origin and the potential planting sites. Seedlings from the riverine habitat types are unlikely to perform well in arid and semi arid areas. Planting some dry upland sources under optimum conditions may on the other hand result in slow growth rate.

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