Calliandra calothyrsus

An agroforestry tree for the humid tropics

Edited by
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Institutions

CATIE	Centro Agronómico Tropical de Investigación y Enseñanza, Turrialba, Costa Rica
CIAT	Centro Internacional de Agricultura Tropical, Cali, Colombia
CSIRO	Commonwealth Scientific and Industrial Research Organisation, Canberra, Australia
DFID	Department for International Development, UK
ESNACIFOR	Escuela Nacional de Ciencias Forestales, Siguatepeque, Honduras
FAO	Food and Agriculture Organisation of the United Nations, Rome, Italy
FPRI	Forest Products Research Institute, Bogor, Java, Indonesia
FSP	Forages for Smallholders Project, CIAT
GTZ	Deutsche Gesellschaft für Technische Zusammenarbeit, Eschborn, Germany
ICRAF	International Centre for Research in Agroforestry, Nairobi, Kenya
IUCN	International Union for the Conservation of Nature
KEFRI	Kenya Forestry Research Institute
KWDP	Kenya Woodfuel Development Programme
NifTAL	Nitrogen Fixation for Tropical Agriculture, University of Hawaii, USA
NRC	National Research Council, Washington D.C., USA
OFI	Oxford Forestry Institute, UK

Terminology

ADF	Acid detergent fibre
CT	Condensed tannin
DM	Dry matter
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphates
HT	Hydrolysable tannins
IBA	Indole butyric acid
IVDMD	*In vitro* dry matter digestibilities
MPT	Multipurpose tree
NDF	Neutral detergent fibre
PCR	Polymerase chain reaction
PEG	Polyethylene glycol
RAPD	Random amplified polymorphic DNA
SALT	Sloping Agricultural Land Technology
VAM	Vesicular-arbuscular mycorrhizal fungi
VFC	Voluntary feed consumption

Acknowledgements

1   Introduction

Calliandra calothyrsus Meisn. is a small, neotropical tree that in recent years has been the focus of substantial research and cultivation. While the predominant use of *C. calothyrsus* is increasingly the production of fodder for ruminant livestock, other uses are also found within different farming systems and include the provision of green manure, fuelwood, shade (traditionally for coffee, but also for tea), land rehabilitation, erosion control, and honey production. The species has particular relevance in the humid tropics and on acidic soils where other agroforestry trees perform poorly. It is primarily used outside its native range, in areas where high population density places pressure on limited land resources, and where agroforestry systems are being developed to maintain and sustain agricultural production.

The history of use of *C. calothyrsus* almost certainly extends back before the date of the first botanical collection of the species by Kegel in 1846. Kegel's specimen was collected from hillsides beside the river Mariepastonkreek in Surinam (Meisner, 1848). Surinam falls outside the natural range of the species and this population must have been introduced, either as an ornamental by virtue of its showy red flowers, or for some other product. Moreover, the fact that descendants of the Mayans from Bonampak, Mexico still eat the green seeds of *C. calothyrsus* has generated speculation that it may have been used very much earlier than the 19th century (Macqueen, 1993).

The first written records of the species' use date from 1936 when it was introduced from Guatemala to Java, Indonesia, by the Dutch colonial forestry service as a replacement for *Leucaena leucocephala* in higher altitude, humid locations where the latter species' heavy seed production and competitive root growth were considered problematic (Verhoef, 1939; 1941). Recent isozyme-based analyses, on populations of *C. calothyrsus* from both the native range and exotic environments, have shown that the origin of introductions to Indonesia was most probably from the vicinity of Santa María de Jesus in the state of Quezaltenango, Guatemala (Chamberlain, 1996a; 1998a; section 7.3). After initial plantings in forestry trial plots and as shade for coffee in east Java, the species' promising growth rate encouraged further research work, but progress was interrupted by World War II (NRC, 1983; Kartasubrata, 1996; Riswan *et al.*, 1996).

After the war, research into site requirements, germination and establishment of *C. calothyrsus* continued (Hellinga, 1950; Sjafei, 1959). In 1974, the Indonesian State Forest Corporation, Perum Perhutani, used 1,700 forest guards scattered throughout rural Java to distribute *C. calothyrsus* seedlings so that villagers could meet their fodder and fuelwood needs. Trial plots had demonstrated that the species was suited to this purpose in humid areas and at middle elevations (250-800m; NRC, 1983). In a collaborative programme involving forest guards and villages (the MA-LU programme), village chiefs were sufficiently impressed by the trial plots to support the establishment of quarter-hectare, temporary nurseries and to encourage the distribution of *C. calothyrsus* throughout Java. By 1983, an estimated 170,000 ha of the species had been planted in Java alone (NRC, 1983), and by 1996 the total in Indonesia had risen to between 500,000 and 1,000,000 ha (Silitonga, 1996).

By the early 1980s, interest in the species had arisen in other areas of the humid tropics. In the species' native range, the Centro Agronómico Tropical de Investigación y Enseñanza (CATIE), Costa Rica, had initiated trials to examine biomass production from line plantings of *C. calothyrsus*, the seed of which came from Indonesia (Baggio and Heuveldop, 1984). In 1984, CATIE collected seed from 11 provenances in Guatemala and Costa Rica for evaluation in a series of Central American trials (Chang and Martinez, 1984).

Outside the native range, *C. calothyrsus* (of unspecified origin) was established in 1981 at Bangalore, Karnataka, India for biomass trials (Kushalappa, 1989). By 1982, seed had been distributed to Western Samoa for inter-cropping experiments (Kidd and Taogaga, 1984), to Taiwan for seedling experiments (Hu *et al.*, 1983) and to Thailand for species evaluation trials (Yantasath *et al.*, 1985). In 1983, *C. calothyrsus* (again from an unspecified source) was established in Hawaii for wood volume prediction trials (MacDicken and Brewbaker, 1983). In the same year, the first introductions of *C. calothyrsus* to Africa took place for agroforestry evaluation in alley cropping systems in Nigeria (Gichuru and Kang, 1989), and by 1984, seed had also been introduced to Kenya (Nyamai, 1987). In 1985, the first major fodder evaluation programme of the species began at the University of Queensland, Australia (Shelton *et al.*, 1996).

By 1990, the list of countries actively involved in research on *C. calothyrsus* had risen to 20, including, in addition to those listed above, Bolivia, China, Colombia, Guinea, Haiti, Jamaica, Papua New Guinea, Philippines, Rwanda, Sri Lanka and Zimbabwe. It is likely that seed for such experimentation came from

Indonesia, since CATIE's germplasm was confined to use in Central America. Oxford Forestry Institute (OFI) also maintained a small seed collection of *C. calothyrsus* representing five provenances that had been first distributed in 1989 (Macqueen, 1992).

The use of seed from a potentially narrow genetic base raised fears that the species might be vulnerable to pest or disease attack, as happened with *Leucaena leucocephala* (Hughes, 1998). In addition, the limited number of provenances evaluated in trials restricted the potential for future genetic selection. As a result, in 1990, the Oxford Forestry Institute initiated a project funded by the UK Government's Department for International Development (DFID, formerly ODA) to explore and collect seed of the species throughout its native range (Macqueen, 1991). Seed collections from 50 provenances were completed by 1993, and in the same year pilot trials were established in five countries, including first-time introductions to Brazil, Cameroon and New Caledonia (Pottinger, 1993).

By 1996, OFI *C. calothyrsus* seedlots had been sent to 39 countries (Pottinger, 1996a). The culmination of this trial series was the formation of an official *Calliandra* trial network at the International Workshop on the Genus *Calliandra* held in Bogor, Indonesia in 1996 (Pottinger, 1996b). The objective of this network was to enhance collaboration and communication between scientific and extension groups working on research, development and promotion of the species for the benefit of rural communities around the world.

The rapid recent growth in exotic plantations and results of the trial network has stimulated demand for seed of high performing provenances. Effective seed production methodologies require a detailed knowledge of the reproductive biology of *C. calothyrsus*. Prolonged and abundant flowering is typical of the species, leading many researchers to believe that trees should produce large quantities of seed (Boland and Owour, 1996). This is rarely the case, however, and low seed yields in seed production orchards have been regularly reported with little understanding why this might be so (Chamberlain and Rajaselvam, 1996). Poor seed production in *C. calothyrsus* is a major limitation to the further use of the species.

The purpose of this Tropical Forestry Paper is to provide a summary of the current state of knowledge with regard to *C. calothyrsus*. As indicated above, research on the evaluation and use of the species has been substantial. This has been coupled with research into the taxonomy, biology, and genetic diversity of the species and, whilst providing a wealth of information, has also highlighted the limitations of *C. calothyrsus* and areas for further research and evaluation. Essential background information on *C. calothyrsus* is provided

by exploring the taxonomic status of the species (Chapter 2), and its reproductive biology (floral phenology, sexual system, breeding system, pollination and inter-specific hybridisation) (Chapter 3). The issues discussed in these chapters form the foundation for more applied studies on seed collection and propagation of *C. calothyrsus* (Chapter 4), the use and management of the species (Chapter 5) and a description of the results of the *C. calothyrsus* provenance trials (Chapter 6). An insight into the way 'purer' research can provide the basis for more applied studies is illustrated by the use of molecular marker techniques (Chapter 7). Lastly, Chapter 8 focuses on conservation priorities for the species. In each chapter, the main findings are summarised into practical recommendations for the improved use of the species.

Research on *C. calothyrsus* continues to flourish, seeking to address our unanswered questions surrounding the species and its use. It is hoped, however, that this publication will be a useful summary of the current state of our knowledge with regard to *C. calothyrsus*, and that it will be utilised by students and researchers seeking basic information on the species, as well as professional foresters and agronomists seeking advice on ways in which *C. calothyrsus* can be used and managed.

2   Taxonomy

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2.1   *Calliandra* Benth.

The genus *Calliandra* was first described by Bentham in 1844 and placed in the tribe *Ingeae* of the Mimosoid legumes (Bentham, 1875; Elias, 1981; Nielsen, 1981). The *Ingeae* were delimited within the family *Mimosoideae* by calyx shape and large numbers of stamens (more than 10) fused at their base. There has been some speculation as to whether *Calliandra* is best placed in the *Ingeae, Acacieae* or *Mimoseae*, but without further resolution, the current placement in the *Ingeae* is preferred (Macqueen, 1996). Bentham delimited the genus *Calliandra* based on its uniform pod dehiscence and floral characters. Subsequent authors have also stressed the importance of pollen characters in defining the genus (Barneby, 1998; Guinet, 1981). Hernández (1986, 1989) separated a new genus, *Zapoteca* Hernández (comprising 18 species from the southwest United States to Argentina) from *Calliandra*, based in part, upon differences in pollen characteristics.

The genus is native to North, Central and South America with two outlying species in continental Africa (Thulin *et al.*, 1981), nine named from Madagascar (Viguier, 1944) and two from the Indian subcontinent (Paul, 1979). In his recent revision of *Calliandra*, Barneby (1998) recognised 132 species from the Americas, assigned to five sections and 14 series. The centre of diversity within the genus is believed to be in the state of Bahia, Brazil, with a secondary centre of diversity in southern Mexico. Despite the genus' large size, *Calliandra* contains only a few species that are widely utilised; some species are planted as garden ornamentals (e.g., *Calliandra surinamensis* Benth. and *Calliandra haematocephala* Hassk.), but only one species, *Calliandra calothyrsus* Meisn., has been widely cultivated and used within agroforestry systems.

Characteristics of the genus

Calliandra are usually shrubs or small trees, and rarely herbs or large trees. Although frequently inhabiting the forest understorey, often along river banks, a minority of species reach the canopy (e.g., *Calliandra laxa* var. *stipulacea* (Benth.) Barneby reaches 25 m). While many species are found in humid lowland forest, a number occur in montane regions (e.g., *Calliandra hirsuta* (G. Don) Benth.), and some inhabit semi-arid or even arid grassland (e.g., *Calliandra californica* Benth. and *Calliandra gilbertii* Thulin & Hunde). The majority of species are thornless, although notable exceptions occur outside of the Americas (e.g., *Calliandra umbrosa* (Wall.) Benth. from India).

There are a number of features that clearly define the genus *Calliandra* within the *Mimosoideae*. Firstly, fruits are linear or slightly curved, flattened pods with thickened margins. Pods of all species dehisce explosively from apex to base. This type of dehiscence is only found outside of *Calliandra* in a few Australian *Acacia* species (*Acacieae*) (Bentham 1875; Maslin, 1982), in *Calliandropsis* (Hernández and Guinet, 1990), *Alantsdilodendron* (Luckow, 1995; Villiers, 1994), *Gagnebina* (Lewis and Guinet, 1986), *Pseudoprosopis* (Gunn, 1984) and *Zapoteca* (Hernández, 1989), and is then associated with very different floral or pollen characteristics.

The inflorescences of *Calliandra* consist of flowers clustered either in axillary heads or in elongated racemes. The calyx is cup-shaped and the corolla bell-shaped with petals and sepals arranged regularly in almost all cases. The staminal filaments are numerous, united at their base, and at least twice the length of the corolla. In most species, a nectariferous disk is present at the base of every flower. In the species which have been most extensively studied, the principal pollinators are moths and bats, although Arroyo (1981) noted that the flowers of *Calliandra riparia* Pittier are clearly adapted for hummingbird pollination. The leaves of *Calliandra* species are bipinnate with one to many pairs of pinnae, the petiole and rachis usually without nectariferous glands (a feature common to some other genera in the *Ingeae*, e.g., *Inga* Mill.). In the single species, *Calliandra hymenaeodes* (Persoon) Benth., the leaves are pinnate, a feature previously regarded as diagnostic of the genus *Inga*. In *Calliandra*, there are one to many pairs of opposite leaflets without noticeable leaf stalks.

In terms of related genera within the *Ingeae*, *Calliandra* is closest to *Zapoteca*, confirmed by a recent cladistic analysis by Grimes (1995). Other authors (e.g., Guinet and Hernández, 1989), however, feel that *Calliandra* is "...a very isolated genus within the *Mimosoideae*" and "...are unable for the moment even to suggest a generic affinity".

2.2   Series *Racemosae*

Bentham (1875) divided the genus *Calliandra* into five series based on leaf and inflorescence characters: *Macrophyllae, Laetevirentes, Pedicellatae, Nitidae* and *Racemosae*. Series *Racemosae* was delimited on the basis of its species: many pairs of pinnae; numerous pairs of small leaflets; elongated, racemose, paniculate inflorescences; and compact few-flowered floral heads. Series *Racemosae* consists of seven species: *Calliandra houstoniana* (Mill.) Standley, *Calliandra calothyrsus* Meisn., *Calliandra longepedicellata* (McVaugh) Macqueen & H.M. Hern., *Calliandra grandiflora* (L'Hér) Benth., *Calliandra juzepczukii* Standley, *Calliandra palmeri* S. Wats. and *Calliandra physocalyx* H.M. Hern. & M. Sousa (Macqueen, 1993, 1996; Macqueen and Hernández, 1997).

The seven species in Macqueen and Hernández's (1997) series *Racemosae* are coextensive with five species recently revised by Barneby (1998), and placed in a North American group of taxa in series *Calliandra* (Table 2.1). Barneby (1998) recognised the following species: *Calliandra houstoniana* (Mill.) Standley with five varieties (var. *houstoniana*, var. *calothyrsus* [Meisn.] Barneby, var. *acapulcensis* [Britton & Rose] Barneby, var. *anomala* [Kunth] Barneby, and var. *colomasensis* [Britton & Rose] Barneby), *Calliandra juzepczukii* Standley, *Calliandra palmeri* S. Wats., *Calliandra physocalyx* H.M. Hern. & M. Sousa and *Calliandra wendlandii* Benth..

The definition of these taxa, for the most part, run parallel. Barneby conducted his revision within the framework of the whole genus, whereas Macqueen and Hernández focused only on Bentham's series *Racemosae*. Any conflict between the two sets of definitions therefore results from the different interpretation placed on the large amount of morphological variation in the group, which may be further complicated by hybridisation. Barneby concluded, however, that practitioners should utilise Macqueen and Hernández's revision for the taxa of economic importance to avoid unnecessary confusion.

The following descriptions of series *Racemosae* and *C. calothyrsus* are therefore based on the revision of Macqueen and Hernández (1997).

Characteristics of series *Racemosae*

Each member of the *Racemosae* displays terminal inflorescences bearing small pale flowers with showy staminal filaments, red at the tip and white or pink at the base (very rarely white throughout). The inflorescences are elongated paniculate racemes (to 40 cm long) which open nocturnally from base to tip over a period of months with only a few flowers open on any one night. Individual flowers are clustered in sub-umbels on short peduncles. Flowers are borne on a short stalk (or pedicel) and have cup-shaped toothed calyces and corollas. In some species (e.g., *C. calothyrsus*), some flowers within the inflorescence are functionally male (staminate) and are without a pistil. Pollinators are bats or large moths, although the flowers are visited for nectar by humming birds, bees and other insects which do not come into contact with the anthers (Chamberlain and Rajaselvam, 1996; Hernández, 1991).

Table 2.1:   Recent taxonomic revisions of Bentham's series *Racemosae* (1875).

Macqueen and Hernández (1997)	Barneby (1998)
C. houstoniana subsp. *houstoniana*	*C. houstoniana* var. *houstoniana*
C. houstoniana subsp. *alamosensis*	*C. houstoniana* var. *houstoniana*
C. houstoniana subsp. *stylesii*	*C. houstoniana* var. *houstoniana*
C. calothyrsus	*C. houstoniana* var. *calothyrsus*
C. calothyrsus	*C. houstoniana* var. *acapulcensis*
C. grandiflora	*C. wendlandii*
C. grandiflora	*C. houstoniana* var. *colomasensis*
C. grandiflora	*C. houstoniana* var. *anomala*
C. longpedicellata	*C. houstoniana* var. *anomala*
C. juzepczukii	*C. juzepczukii*
C. palmeri	*C. palmeri*
C. physocalyx	*C. physocalyx*

Fruits develop up from the base to the tip of the inflorescence axis and are flattened pods with raised margins which open explosively propelling seeds up to 10 m from the maternal parent. In some species, pods are covered in dense hairs (as are plant stems and leaves, e.g., *C. houstoniana*), whilst in other species hairs are sparse or absent.

Species within series *Racemosae* are native to Mexico and Central America. They are all bushes or small trees. The leaves within series *Racemosae* have between three and 26 pairs of pinnae and numerous pairs of small leaflets. The stipules at the base of the leaves are ephemeral and vary in shape from lanceolate to oblanceolate between species.

A key to the species within the *Racemosae* is given by Macqueen and Hernández (1997).

2.3   *Calliandra calothyrsus* Meisn.

Calliandra calothyrsus *Meisn.* in Linnaea 21: 251 (1848); Benth. in Trans. Linn. Soc. London 30: 556 (1875); McVaugh in Fl. Nov. Galiciana 5: 153 (1987); H.M. Hernández in Anales Inst. Biol. Univ. Nac. Autón. México, Ser. Bot. 62: 121-132 (1991); Macqueen, Commonw. Forest. Rev. 71: 20-34 (1992). Type: Surinam, Mariepaston, *Kegel* 1465, 1846 (holotype NY!; isotype GOET).

Synonyms

Anneslia calothyrsus (Meisn.) Donn.-Sm. in Enum. Pl. Guat. 1: 10 (1889).

Feuilleea calothyrsa Kuntze in Rev. Gen. Pl. 1: 187 (1891).

Calliandra confusa Sprague & L. Riley in Bull. Misc. Inform., Kew 1923: 371 (1923); Standl. in Field Mus. Nat. Hist., Bot. Ser. 10: 283: 210 (1931); Standl. in Field Mus. Nat. Hist., Bot. Ser. 12: 350: 159 (1936); Standl. in Publ. Field Mus. Nat. Hist., Bot. Ser. 18: 392: 492 (1937); Standl. & Steyerm. in Fieldiana, Bot. 24, 5: 22--23 (1946); Woodson & Schery in Ann. Missouri Bot. Gard. 37: 250 (1950). Type: Guatemala, Alta Verapaz, Cobán, *Tuerckheim* 690, (holotype K!; isotype NY!).

Anneslia confusa (Sprague & Riley) Britton & Rose in N. Amer. Fl. 23: 70 (1928).

Calliandra similis Sprague & L. Riley in Bull. Misc. Inform., Kew 1923: 372 (1923); Standl. in Publ. Field Mus. Nat. Hist., Bot. Ser. 18: 392: 493 (1937). Type: Costa Rica, San José, San José, *Oersted* 56, 1857, (holotype K!).

Anneslia similis (Sprague & L. Riley) Britton & Rose in N. Amer. Fl. 23: 71 (1928).

Anneslia acapulcensis Britton & Rose in N. Amer. Fl. 23: 72 (1928). Type: Mexico, Guerrero, Acapulco, *Palmer* 59, 1894 (holotype NY!; isotype US!).

Calliandra acapulcensis (Britton & Rose) Standl. in Field. Mus. Nat Hist., Bot. Ser. 11: 159 (1936).

Botanical description

Shrubs or small trees, single or multiple stemmed, to 12 m tall; stems to 20 cm diameter at base; branchlets terete, striate, occasionally angulate, brown, occasionally red, glabrescent to shortly white-pilose. Pinnae (3-) 6-14 (-19)-jugate; petioles (4-) 16-25 (-38) mm long, terete to angulate; rachis (50-) 80-130 (-190) mm long, terete or angulate, pilose, particularly along the groove borders; rachillae 30-101 mm, the ridge sparsely red- or white-pubescent; leaflets (11-) 19-66 pairs per pinna, 3-10 x 0.5-3 mm, linear-oblong, almost straight, oblique at base, acute or obtuse, membranous, pale to mid green adaxially, paler abaxially, glabrous to sparsely ciliate, midrib subcentric. Stipules to 4 x 1 mm, triangular-lanceolate, rounded at base, acuminate apically, ciliate. Inflorescence to 17 cm long, 4-5 (-6) flowers per umbel; peduncle 5-10 mm long; pedicel 2-5 mm long at anthesis; calyx (1-) 1.5-3 mm long, membranous, glabrous or very slightly pubescent, ciliate, pale yellow green, with obtusely triangular teeth, to 0.7 mm; corolla widely campanulate, 6-8 mm long, membranous, glabrous or very slightly pubescent, pale green, occasionally with purple tinges, with acute lobes, 3-5 mm long; filaments 5.5-6.5 cm long, white in basal half, pink-red in distal half to pink just at base, bright red for the distal portion, tube 2-3 mm; ovary 2-3 mm long, sessile, glabrous; style 6.5-7.5 cm long; stigma cup-shaped. Pods to 142 mm x 180 mm, occasionally rostrate, thickly membranous, pale to dark brown, glabrous, rarely densely pubescent with multicellular conical or filiform capitate hairs. Seeds 8 (-12), ovate, 7-8 x 5-6 mm, brown, dark mottled. Seedling phaneroepigeal, first eophyll pinnate, second eophyll bipinnate, alternate. Base chromosome number $\underline{n} = 11$ (Figure 2.1).

Distribution

Calliandra calothyrsus is found from the western Pacific coast of Mexico at Colima (19°04'N 103°45'W), with an outlying population in Veracruz, Mexico (19°20'N 96°20'W), to the north coast of central Panama (9°20'N 79°50'W) (Figure 2.2; Macqueen and Hernández, 1997). It is found in each of the intervening countries and in the states listed below:

- ♦ Mexico (Chiapas, Guerrero, Michoacan, Oaxaca, Veracruz,

- Guatemala (Alta Verapaz, El Progreso, Guatemala, Huehuetenango, Izabal, Petén, Quiché, Retalhuleu, Sacatepéquez, Santa Rosa, Sololá, Suchitepéquez)

- Belize (Belize, El Cayo, Stann Creek, Toledo)

- Honduras (Atlántida, Colón, Comayagua, Copán, Cortés, El Paraíso, Francisco Morazán, Intibuca, Ocotepeque, Olancho, Santa Barbara)

- El Salvador, (Ahuachapán, Santa Ana)

- Nicaragua (Boaco, Chontales, Estelí, Grenada, Jinotega, Madriz, Managua, Matagalpa, Nueva Segovia, Zelaya)

- Costa Rica (Alajuela, Cartago, Guanacaste, Heredia, Limón, Puntarenas, San José)

- Panama (Chiriquí, San Blas)

Habitat

Calliandra calothyrsus occurs in primary, secondary or disturbed, lowland to pre-montane, seasonally dry to wet sub-tropical forests, especially along river margins. It is tolerant only of medium shade. It occurs on a range of often acidic soils (alluvial deposits, clays, sandy loams; pH 5-6.5) of various depths. It is not tolerant of frost, and requires a mean annual temperature of 22-28°C. The species inhabits areas with an annual rainfall of 700-4000 mm at altitudes between 0-1850 m above sea level.

Common names

Cabello de angel, Pelo de angel, Cabellito, Barbe jolote, Barbe sol, Barbillo, Clavellino (Spanish); Kalliandra, Kalliandra merah (Indonesian); Calliandra (English).

2.4   Variation within *C. calothyrsus*

Calliandra calothyrsus is a wide-ranging, highly variable species. Four sub-groups within the species have been recognised from geographically-based patterns of morphological and isozyme variation (Table 2.2; Chamberlain, 1996a; 1998a; Chamberlain *et al.*, unpublished results).

Basis for the delimitation of sub-groups

Descriptions of the morphological variation within *C. calothyrsus* noted that there were certain geographical patterns that corresponded to morphological trends in flower size, filament colour, pod pubescence, length of petioles and rachilla, and leaflet size (Macqueen, 1996; Macqueen and Hernández, 1997). Macqueen and Hernández (1997) observed that there was a tendency for populations north west of the Isthmus of Tehuantepec, Mexico, to have white bark, small flowers, more white at the base of the filaments, and small pods with short white pubescence. Trees with a similar morphology were also found in dry regions of central Honduras and Nicaragua. Populations to the south east of the Isthmus of Tehuantepec tended to have red-brown bark, large flowers, red filaments, and glabrous pods. On the south coast of Guatemala populations of unusually large trees with large leaves occurred, often with marked red colouring on the leaf axis and petiole. In Costa Rica and Panama, and in southern Guatemala, populations tended to have leaves with high numbers of pairs of pinnae.

An analysis of isozyme variation in the species (Chamberlain, 1996a; 1998a) suggested that there were four groups of populations within *C. calothyrsus* from four geographical regions: south east Mexico, Guatemala and the north coast of Honduras (*C. calothyrsus-1*); Costa Rica and Panama (*C. calothyrsus-2*); the dry inland valleys of Honduras and Nicaragua (*C. calothyrsus-3*); and the Pacific coast of Mexico (*C. calothyrsus-4*). Populations representing *C. calothyrsus-3* and *–4* were found to be more similar to one another than to populations representing *C. calothyrsus-1* and *–2*. Chamberlain *et al.* (unpublished results) also described the morphological differences between these groups with the use of multivariate analyses, which corresponded closely to the description of isozyme variation within *C. calothyrsus*.

Support for the division of sub-groups within *C. calothyrsus* also comes from the results of fodder quality analyses carried out in Australia (Palmer and Ibrahim, 1996). Certain populations were found to be more acceptable to cattle, with generally high figures for percentage nitrogen, high dry matter digestibility and high tannin concentration. Such populations were all from south east Mexico, Guatemala, Belize, the north coast of Honduras, or from Costa Rica (i.e., *C. calothyrsus-1* and *-2*). Populations analysed from the inland valleys of Honduras and Nicaragua (i.e., *C. calothyrsus-3* and *-4*) all had a low rank for percentage nitrogen, dry matter digestibility and tannin concentration.

Table 2.2: Division of *Calliandra calothyrsus* into four subgroups based on geographical, morphological and isozyme characters (after Chamberlain,1998a; Chamberlain *et al.*, unpublished results).

Character	C. calothyrsus-1	C. calothyrsus-2	C. calothyrsus-3	C. calothyrsus-4
Geography				
Location	North coast of Honduras, Belize, El Salvador, Guatemala, Chiapas, Mexico, with an outlying population in Veracruz, Mexico.	Costa Rica and Panama	North west of Costa Rica, Nicaragua and central western Honduras.	Pacific coast of Mexico to the north of Tehuantepec Isthmus.
Ecology				
Altitude (m a.s.l.)	0-1850	0-1200	300-1100	0-240
Mean annual rainfall (mm)	1500-4000	2000-4000	750-3000	750-1450
Length of dry season (months)	1-3	1-3	2-7	7
Morphology				
Tree height	To 12 m	2-8 m	2-8 m	2-10 m
Bark colour	Red-brown	Dark brown	Grey to pale brown	Grey to pale brown
No. of pinnae	12-19	12-19	6-12	6-12
No. of leaflets	27-66	27-66	15-51	15-51
Inflorescence type	Elongated	Elongated	Compact	Compact
No. of staminal filaments	32-66	32-66	16-38	16-38
Length of white basal portion of filament	Less than 10 mm	Less than 10 mm	More than 10 mm	More than 10 mm
Pod length	12-15 cm	8-12 cm	7-14 cm	6-12 cm
Pod pubescence	Glabrous	Glabrous, occasionally with dark hairs	Sparse to dense short white hairs	Sparse to dense short white hairs
Isozyme markers				
Fixed isozyme alleles	*Mdh*-1b	*6-Pgd*-3b	*Mdh*-4b	*Mdh*-1c

Description of *Calliandra calothyrsus-1* and *-2*

Shrubs or trees to 12 m tall. Stems often markedly angulose, green or tinged with red. Bark dark brown or red-brown. Leaves mid-green. Pinnae (7)-12-19-jugate. Leaflets (11) 27-66 pairs; 6-13 times longer than they are wide; width 0.2-1.0 mm. Inflorescence elongated to 17 cm long. Flowers with numerous staminal filaments (29)-32-66. White basal portion of staminal filaments less than 1 cm long. Pods glabrous or with sparse dark hairs.

Populations within this sub-group occur in riverine sites or disturbed forest from central Panama to the state of Chiapas, Mexico, with an outlying population in Veracruz, Mexico. They inhabit areas with an annual rainfall of between 1500-3800 mm with 0-4 months dry season (i.e., with less than 60 mm of rain per month), and occur at altitudes between 0-1850 m above sea level.

Considerable morphological and isozyme variation occurs throughout the range of this sub-group. Much of this variation surrounds two geographically separated types. The northern type (*C. calothyrsus-1*, which relates to Sprague and Riley's *C. confusa*; 1923) occurs from the north coast of Honduras through Belize, El Salvador and Guatemala to Chiapas, Mexico, with an outlying population in Veracruz, Mexico. It occurs in areas with rainfall typically between 1500-3000 mm per annum, at altitudes between 0-1850 m with a short dry season. It is characterised by trees of large stature (up to 12 m tall) with red-brown bark and angular young shoots tinged with red. The flowers are borne on relatively long pedicels (2.7)-3.0-4.6 mm. Mature pods are (10)-12-15 mm wide and glabrous or glabrescent.

The southern type (*C. calothyrsus-2*, which relates to Sprague and Riley's *C. similis*; 1923) is native to Costa Rica and Panama. It inhabits areas with rainfall between 2000-4000 mm per annum, at altitudes between 0-1200 m and with a short dry season. It is characterised by trees that are 2-8 m tall with dark brown bark and round, or slightly angular young stems. The flowers are borne on short pedicels 1.2-2.8-(3.3) mm. Mature pods are 8-12-(13.5) mm wide and glabrous, or occasionally covered in dark hairs.

Description of *C. calothyrsus-3* and *-4*

Shrubs or trees 2-10 m tall. Stems are round or slightly angulose and green or pale brown. Bark is grey or pale brown. Leaves yellow-green. Pinnae (3)-6-12-jugate. Leaflets 15-51 pairs; 3-6 times longer than they are wide; width (0.5)-1.0-3.5 mm. Inflorescence compact, usually less than 10 cm long. Flowers with 16-38-(46) staminal filaments. White basal portion of staminal filaments at least 1 cm long. Pods sparsely to densely short white pubescent. Populations within this sub-group occur by rivers, or more frequently, in disturbed dry forest, e.g., in shifting cultivation or along roadsides. Its native range extends from the far north west of Costa Rica and south east of Nicaragua to the central western border of Honduras, and then from the Tehuantepec isthmus of Mexico northwards along the Pacific coast to Colima. It inhabits areas with an annual rainfall between 750-3000 mm (although usually below 2000 mm), with a 2-7 month dry season (i.e., rainfall below 60 mm per month), and is found at altitudes between 0-1100 m above sea level.

Variation within the sub-group is largely geographically-based and particularly focused around the disjunct northern and southern types. The southern type (*C. calothyrsus-3*) is confined to the far north west of Costa Rica, throughout Nicaragua to central western Honduras. It is found in areas with 900-3000 mm annual rainfall and 2-7 months dry season. It inhabits inland riverbanks and hillsides at altitudes between 300-1100 m. Trees are 2-8 m tall. Leaves have between (18)-35-51 pairs of leaflets. Flowers are borne on pedicels (1)-2-5 mm long. Pods are 7-14 cm long.

The northern type (*C. calothyrsus-4,* which relates to Standley's *C. acapulcensis*; 1936) occurs along the Pacific coast of Mexico to the north of Tehuantepec Isthmus. It inhabits areas with between 750-1450 mm annual rainfall and a long 7-month dry season (with less than 60 mm of rain per month). It is rarely found at altitudes above 240 m and is commonly found close to the coast. Trees are between 2-10 m tall. Leaves have comparatively few leaflets pairs 15-35-(46). Its flowers are borne on short pedicels 0.8-2.0-(3.5) mm long. Pods are 6-12 cm long. In the drier portions of central Chiapas (e.g., close to Ixtapa) intermediate populations exist which fall between those of the two predominant types to the north and south (Chamberlain *et al.*, unpublished results).

Formal botanical descriptions of the sub-groups within *C. calothyrsus* have been avoided for the present. In the light of Barneby's recent revision of the genus *Calliandra* (1998), there maybe some merit in removing *C. acapulcensis* from synonymy with *C. calothyrsus.* These taxa are clearly differentiated by the work of Barneby (1998), Chamberlain (1998a) and Chamberlain *et al*. (unpublished results).
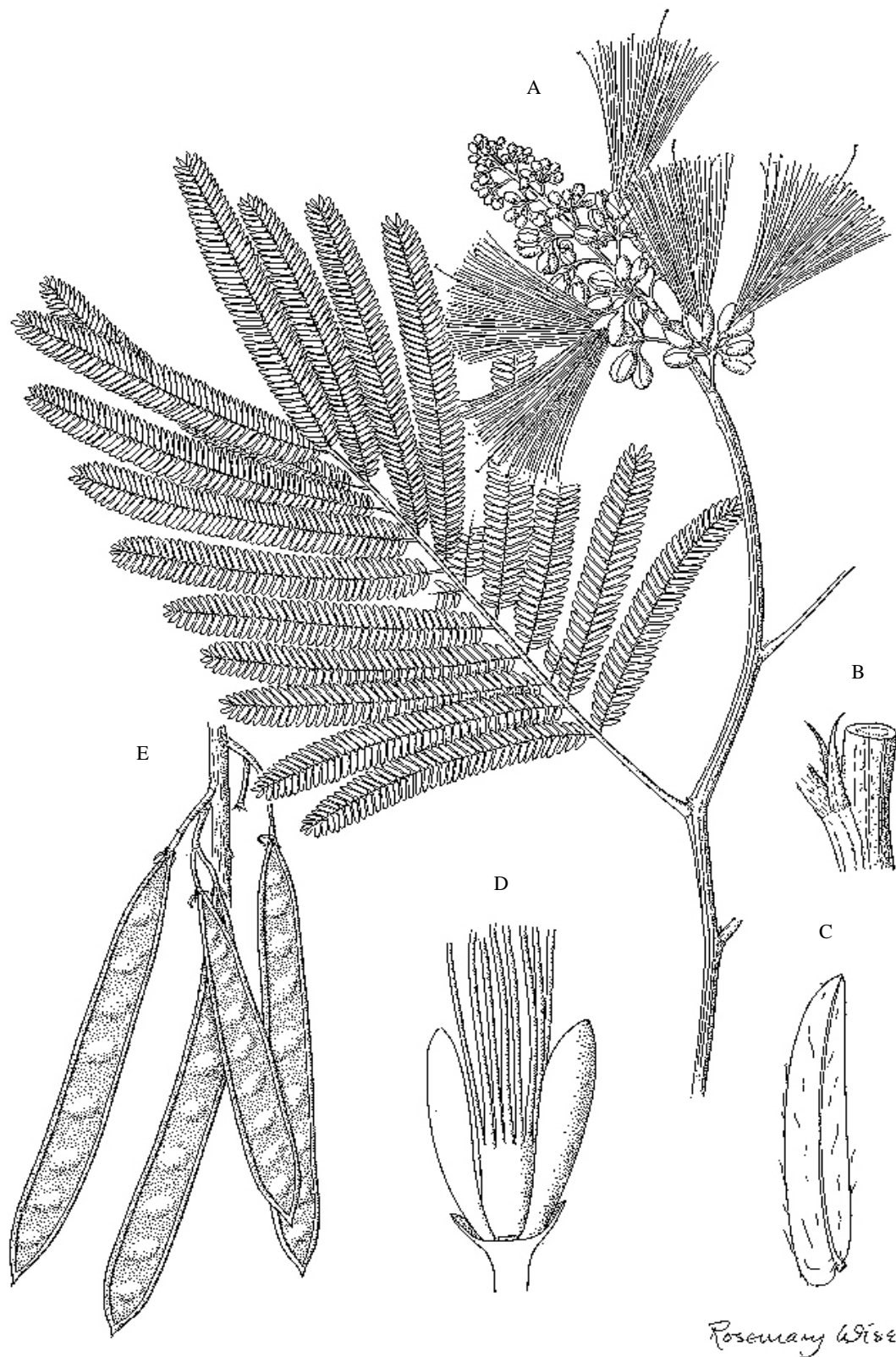
Figure 2.1: *Calliandra calothyrsus* from Guatemala. **A** inflorescence and mature leaf (*Hellin & Hughes 23*); **B** stipule; **C** leaflet; **D** flower transverse section showing staminal tube (*Fulloon 108988*); **E** mature pods (*Hughes 725*). Drawn by Rosemary Wise.
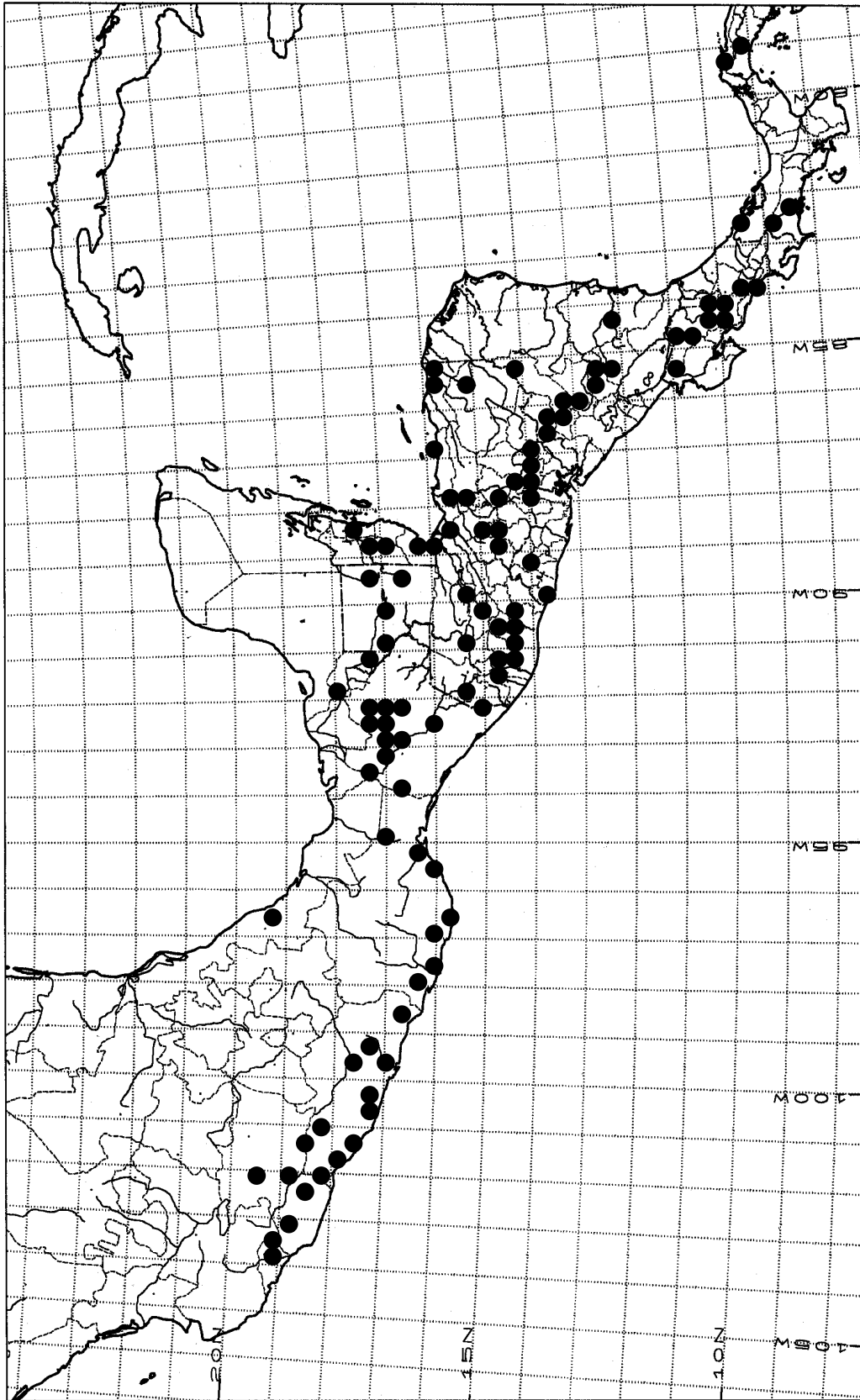
Figure 2.2:   Natural distribution of *Calliandra calothyrsus* in Mexico and Central America (based on herbarium specimens).

Plate 1

A - Inflorescence of *Calliandra calothyrsus* (typical of *C. calothyrsus*-1).

B - Fruiting stem of *Calliandra calothyrsus* showing fruits at various stages of maturity.

C - Fully dehisced fruits of *Calliandra calothyrsus*.

D - Inflorescence of *Calliandra calothyrsus* (typical of *C. calothyrsus*-3).

E - Leaf of *Calliandra calothyrsus* showing it's pinnae and leaflets.

3 Reproductive biology and seed production

J.R. Chamberlain[1] and J.D. Hubert[2]

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3.1 Introduction

A thorough understanding of reproductive biology is critical for the effective conservation, utilisation and management of a plant species. For many plants, particularly tropical trees, knowledge of reproductive biology, i.e., floral phenology, breeding and pollination systems, fructal phenology and seed dispersal, is generally limited (Bawa *et al*., 1990; NRC, 1991). Patterns of flowering will influence the genetic structure of tree populations, and provide information on which to base seed procurement activities. For outcrossing species, the flowering of individuals relative to one another and to pollinator availability affects patterns of mating and gene flow. The breeding system of a plant species, i.e., whether a species is predominantly outcrossing or selfing, affects the partitioning of genetic variation within and between populations, which influences sampling strategies for seed collection and the design of seed production orchards. Pollinators and their mode of behaviour when foraging for pollen and nectar resources similarly affect the genetic structure of populations, and may influence a species' adaptation to exotic environments. Lastly, fructal phenology and the mode of seed dispersal determines the pattern of seed distribution from mother trees, which again affects gene flow and population genetic structure (Hamrick *et al*., 1992).

Studies on the reproductive biology of *C. calothyrsus* have special significance because low seed yields have often been obtained where the species is cultivated in exotic environments. *C. calothyrsus* flowers precociously, but contrary to the expectations of some practitioners, sets relatively little seed in comparison to the abundance of flowers it produces (Boland and Owour, 1996; FSP, 1998; B.M. Kamondo, pers. comm[1].; B. Duguma, pers. comm[2].). One or more of a range of factors may be responsible for this, including low visitation rates by pollinators and/or adverse climatic conditions. The purpose of this chapter is to summarise the results of recent research on the reproductive biology of *C. calothyrsus*, including its implications for seed production in the species. In addition, the potential for inter-specific hybridisation within *Calliandra* series *Racemosae* is discussed.

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3.2 Floral morphology and phenology

Floral morphology

The inflorescence of *C. calothyrsus* is a paniculate raceme with flowers that open acropetally, i.e., from the base to the tip of the inflorescence, over a period of between 60-90 days. The flowers are held in sub-umbels with 4-5 flowers per umbel. The flowers are regular with a fused calyx and corolla, and staminal filaments that are also fused at the base to form a tube 2-3 mm long. The filaments are usually white in the basal portion and pink to red in the distal portion and bear centrally attached anthers. The anthers dehisce outward and downward to reveal the polyads which are eight-grained aggregates of pollen. The style, which is white to pale yellow, extends some 10 mm beyond the staminal filaments and bears a cup-shaped stigma. The number of ovules contained in the ovary varies from 8-13 (Boland and Owour, 1996; Macqueen and Hernández, 1997; Chamberlain, 1998b).

Anthesis normally occurs between 15:30 and 17:00 h (Chamberlain, 1998b; Rajaselvam *et al*., 1996), although in Kenya it was recorded to occur as early as 14:00 h (Boland and Owour, 1996), and is followed by anther dehiscence once the flowers are fully open. The stigma is normally receptive from 19:00 h, but by 06:00 h the following morning receptivity has declined and the flowers begin to wilt (Matthews and Hopkinson, 1998; Chamberlain, unpublished data). The number of flowers produced per inflorescence per day has been variously recorded as 1-34 (Boland and Owour, 1996; Macqueen, 1992; Chamberlain, unpublished data). The standing volume of nectar has been recorded within the range of 20-50 µl per flower with sugar concentrations in the range of 14-20% (Boland and Owour, 1996; Rajaselvam *et al*., 1996; Chamberlain, unpublished data). From pollination to seed maturity, a period of between 90-120 days is required.

Floral phenology

Not surprisingly for such a widespread species, the flowering season of *C. calothyrsus* varies throughout the natural distribution. The flowering period can extend across twelve months if sufficient moisture is available, but is concentrated between October and

January. In Mesoamerica, this is the period after the main wet season but before the onset of the dry season. Populations on the Mexican and Guatemalan Pacific coast tend to flower early with a peak during October and November, while populations to the south in Costa Rica and Panama flower later with a longer peak period (January to March). Where a marked dry season of four to five months is experienced, flowering will often cease completely during this period.

Within-provenance variation in floral phenology

Phenological observations were made within a natural population of *C. calothyrsus* found in the River Bonito, Colón, Honduras (Chamberlain, 2000b), an area that does not experience a true dry season. The population is found scattered along the banks of the River Bonito and its tributaries, and can be divided into four sub-populations. Ten inflorescences on each of ten trees of similar size were selected from each sub-population and the number of flowers per inflorescence recorded over a 30-day period. Flowering reached a peak during late January. The trees in all four sub-populations flowered during the period of study, but exhibited variation in the time of peak flowering and the total number of flowers produced per tree. The trees in three of the sub-populations all experienced a peak flowering period 5-15 days after the study was initiated, whereas the trees in the remaining sub-population reached a peak 20-25 days after the study was initiated. There were highly significant differences between sub-populations for mean flower number per tree over the flowering period, and flowering within each sub-population was found to be highly synchronous.

Between-provenance variation in floral phenology

Phenological observations were made over a period of twelve months on six provenances of *C. calothyrsus* planted in 1992 at the University of Peredeniya Experimental Station, Dodangolla, Sri Lanka (Rajaselvam *et al*., 1996). All the provenances flowered during the twelve-month period and generally exhibited a peak flowering period during September to December (months 5-8), which coincided with the wet season in this region of Sri Lanka (Fig. 3.1). Flower production was generally at a lower level from February to August (months 10-12 and 1-4), which coincided with the onset of the dry season. However, trees from the population at Fortuna (Costa Rica) had a peak flowering period from December through to March (months 8-11). Trees from the population at Turrialba (Costa Rica) also had a later peak flowering period, although the lower number of flowers per

inflorescence per tree exhibited by this provenance over the period of study makes the observation less marked. There were highly significant differences between provenances for mean flower number per tree over the flowering period, the trees from Flores (Guatemala) having the highest number of flowers per tree, and those from Turrialba having the lowest number.

Phenological observations were also made over a period of twelve months on 15 provenances of *C. calothyrsus* planted in a trial in 1993 at Yaounde, Cameroon (Duguma and Mollet, 1996). All the provenances studied flowered during the first six months after establishment, but there were significant differences between provenances for the time taken to anthesis, and the time between anthesis and fruit formation. Provenances from Barillas (Guatemala), Bombana (Mexico) and Trujillo (Honduras) flowered the earliest, whilst provenances from Santa María (Honduras), La Puerta (Nicaragua) and Turrialba (Costa Rica) flowered the latest.

The physiological and genetic basis for flowering in tropical trees is not well understood, hence there could be a range of factors affecting the time of flowering and variation in flower number. Rainfall may, by the release of moisture stress or temperature reduction, lead to the onset of flower production (Opler *et al*., 1976). The peak flowering period that coincides with the rainy season in both native and exotic environments suggests rainfall may be initiating flower production in *C. calothyrsus*. Within-population variation in flowering time and the number of flowers produced also indicate that local site conditions may be influencing the onset of flowering, as well as the relative allocation of resources to vegetative versus reproductive growth. Provenance variation in floral phenology also suggests a genetic component to flowering, which may reflect adaptation to the local environmental conditions at the original collection sites.
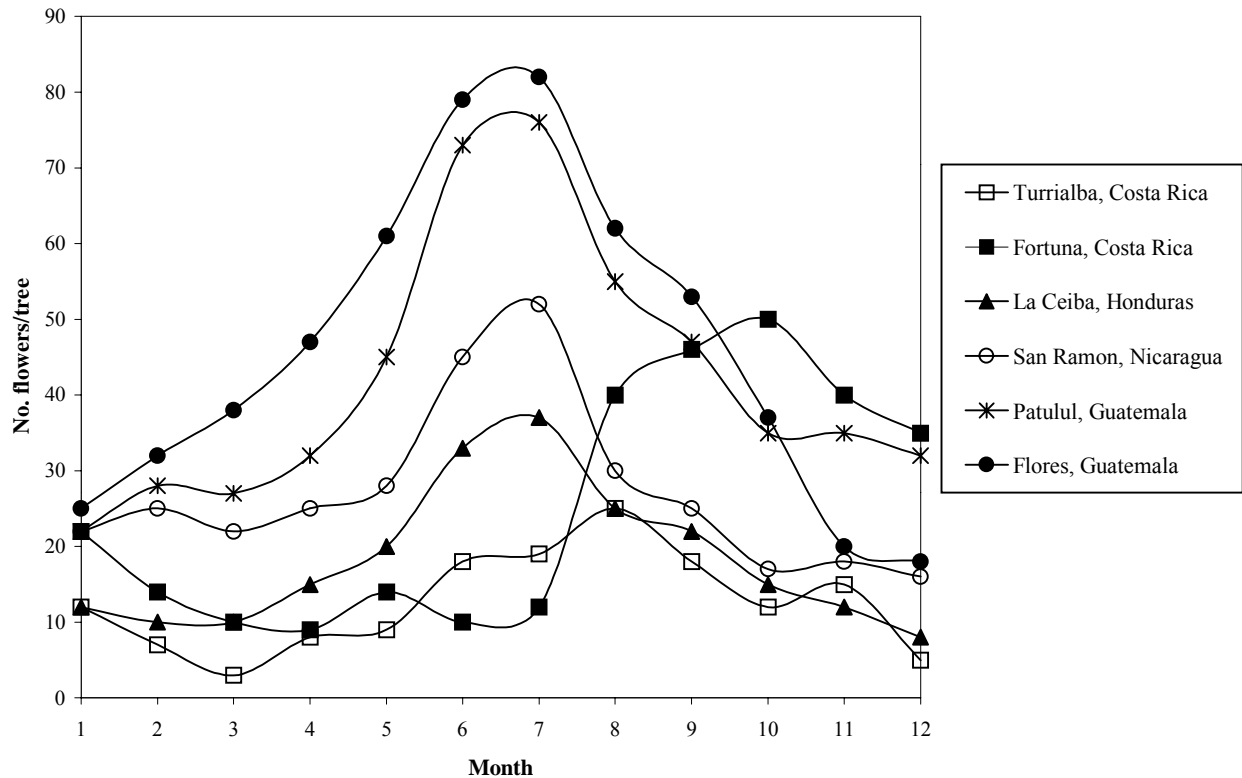
Figure 3.1:   Floral phenology of six provenances of *Calliandra calothyrsus* planted within a trial in Sri Lanka.

The floral phenology of *C. calothyrsus* and its implications for seed production

♦ Anthesis occurs between 15:30 and 17:00 h and is followed by anther dehiscence once the flowers are fully open.

♦ The stigma is receptive from 19:00 h, but by 06:00 h the following morning receptivity has declined and the flowers begin to wilt.

♦ From pollination to seed maturity, a period of between 90-120 days is required.

♦ The peak flowering period in *C. calothyrsus* generally coincides with the period just after the wet season in both native and exotic environments.

♦ There is evidence for between- and within-provenance variation in floral phenology and flower number, which may be influenced by adaptation to local environmental conditions.

Implications: Pollination will only be effected by nocturnal visitors to the flowers. Peak flowering will occur prior to the onset of the dry season, allowing seed to ripen successfully in the subsequent months. There appears to be provenance variation for flower production, hence there may be provenance variation for seed production.

3.3   Sexual system

Calliandra calothyrsus is andromonoecious, i.e., individual trees bear both hermaphrodite (bisexual) and staminate (functionally male) flowers. Staminate flowers, when produced, have been observed either at distal positions on the inflorescence or, when the pods are developing, at a medial position on the inflorescence (Chamberlain, in press). Over a period of nine days, Boland and Owour (1996) recorded the proportion of staminate flowers on trees in a population at Embu, Kenya, to vary from 5-50 percent per inflorescence, but could not relate this to any environmental influences. The staminate flowers are distinguished at anthesis by their completely reduced styles, or withered style remains; however, no differences were observed between staminate flowers and hermaphrodite flowers in terms of the quantity of pollen produced, its viability, or in the flowers' nectar volumes and their sugar concentrations (Chamberlain, in press).

Numerous hypotheses for the evolution and maintenance of andromonoecy can be found in the literature, but current theory suggests that andromonoecy provides a mechanism for optimally allocating limited resources to both the male and female functions (Bawa and Beach, 1981; Bertin, 1982; Charnov, 1982; Diggle, 1993; May and Spears, 1988; Maynard Smith, 1978; Solomon, 1985). The pattern of sex expression exhibited by *C. calothyrsus*, and the plasticity of that pattern in response to varying resource allocation were investigated within a plot of *C. calothyrsus* at the National Forestry School's (ESNACIFOR) Experimental Station, San Juan, Siguatepeque, Honduras (Chamberlain, in press).

The production of hermaphrodite flowers, staminate flowers and pods were monitored over a period of 28 days. All plants produced pods and after 23 days, the mean proportion of staminate flowers had increased and was greater than the mean proportion of hermaphrodite flowers (Figure 3.2).

Within two subsequent experiments, the sex expression of flowers was monitored in the same way, but the plants manipulated so that (a) half the trees in the experiment did not set pods (unpollinated), whilst the remaining half were allowed to set pods as normal (pollinated), and (b) half the inflorescences on an individual tree did not set pods, whilst the other half were allowed to set pods as normal. In this way, the plasticity of sex expression was investigated at the between-tree and within-tree levels.
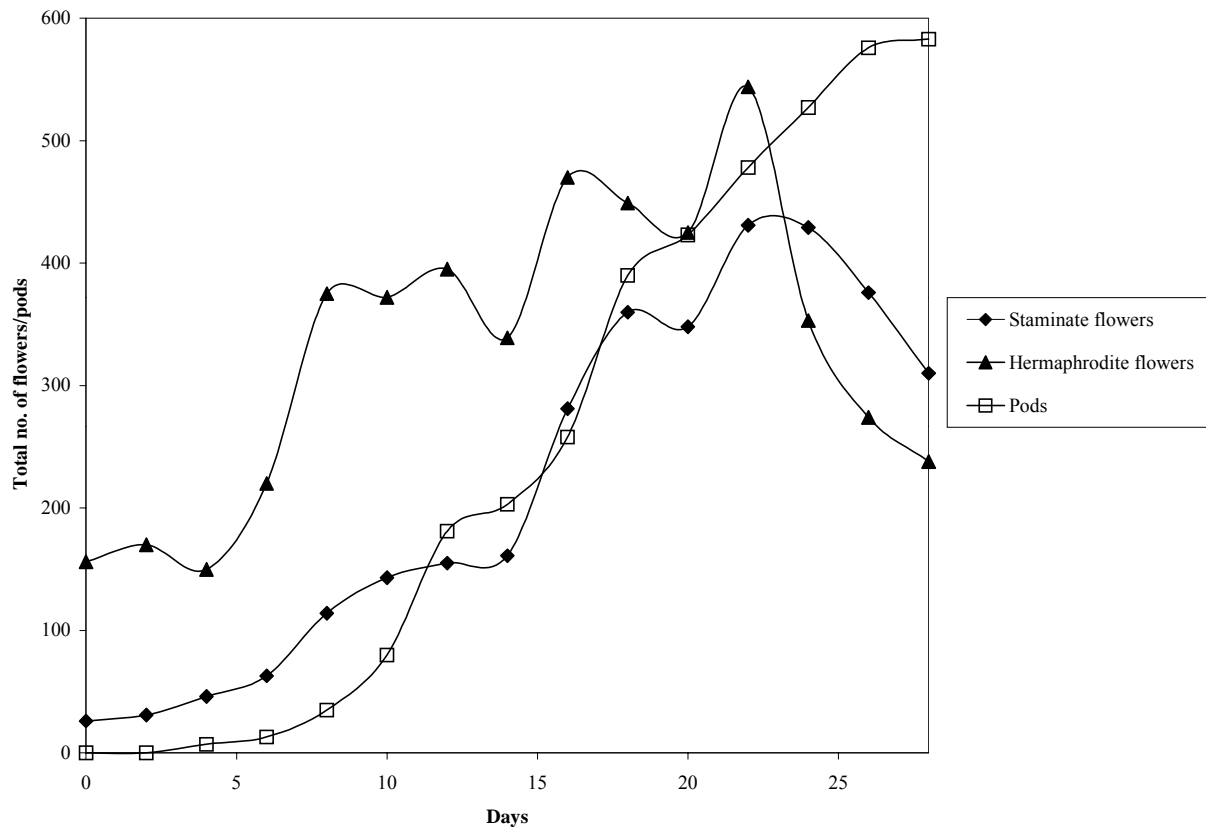
Figure 3.2:   Production of hermaphrodite flowers, staminate flowers and pods over 28 days in a trial plot of *Calliandra calothyrsus* in Honduras.

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In both experiments, the presence of developing fruit had a significant effect on plant sex expression by increasing the mean proportion of staminate flowers per inflorescence on pollinated plants relative to the proportion on unpollinated plants (Figures 3.3a and 3.3b). A striking pattern of sexual allocation along the inflorescence was observed in all the experiments. Once an inflorescence started developing pods in the lower part of the inflorescence, there was a gradual increase in the proportion of staminate flowers towards the apex of the inflorescence. Often a complete switch from hermaphrodite to staminate flower production was observed.

The results suggested that *C. calothyrsus* responds to the production of pods by optimally allocating its resources to both the male and female functions, i.e., through the increased production of staminate flowers, the potential for pollen dispersal is maintained, whilst resources are directed to producing a limited quantity of seed. The effect of the presence of developing pods was also apparent between inflorescences on the same tree, suggesting that resource allocation occurs at the inflorescence level and may be independent of the resources available to the plant as a whole. Inter-inflorescence variation under contrasting conditions of fruit set has been observed in other andromonoecious plants (Diggle, 1994).

The sexual system of *C. calothyrsus* therefore appears to limit the quantity of seed an individual tree can produce. This may be dependent on the resources (nutrients, light, water) available to the plant as a whole, and to individual inflorescences. It has been recommended that the soil in seed production areas is fertilised in order to improve seed yields (Chamberlain, 1996b). In a seed production orchard in Queensland, Australia, the trees are both irrigated and fertilised and the resulting seed yields have been very good (Matthews and Hopkinson, 1998). Although the latter observations are empirical, combined with results from the investigation of the species' sexual system, limited resources are likely to have an important effect on seed production in *C. calothyrsus*.

The sexual system of *C. calothyrsus* and its implications for seed production

♦ *Calliandra calothyrsus* is andromonoecious, i.e., individual trees bear both hermaphrodite (bisexual) and staminate (functionally male) flowers.

♦ The staminate flowers are distinguished by their completely reduced styles, or withered style remains, and cannot be pollinated or go on to produce seed.

♦ The male function, i.e., the production of fertile pollen, is perfectly functional in the staminate flowers.

♦ A striking pattern of sex expression along the length of the inflorescence can be observed in *C. calothyrsus*. Once an inflorescence starts developing pods in the lower part of the inflorescence, there is a gradual increase in the proportion of staminate flowers towards the apex of the inflorescence.

♦ *Calliandra calothyrsus* may be responding to limited resource availability and optimally partitioning those resources between the male and female functions.

Implications: The resources, i.e., nutrients, light, and water, available to the plant as a whole and to individual inflorescences of *C. calothyrsus,* may limit the number of hermaphrodite flowers an individual tree can produce and, therefore, the quantity of seed produced. Fertilisation of a seed production stands may improve seed yields.
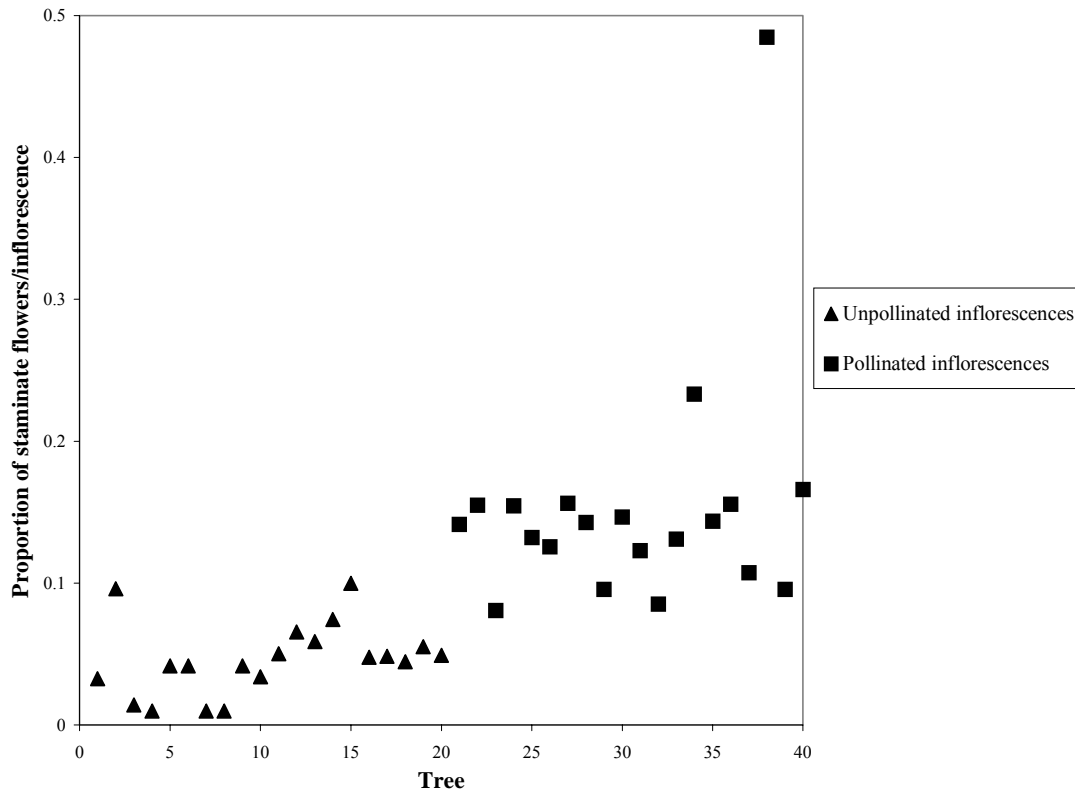
Figure 3.3a: Mean proportion of staminate flowers per inflorescence produced by each of 20 trees in pollinated (plus fruit) and unpollinated (no fruit) treatments during an investigation of whole plant sex expression (Chamberlain, in press).
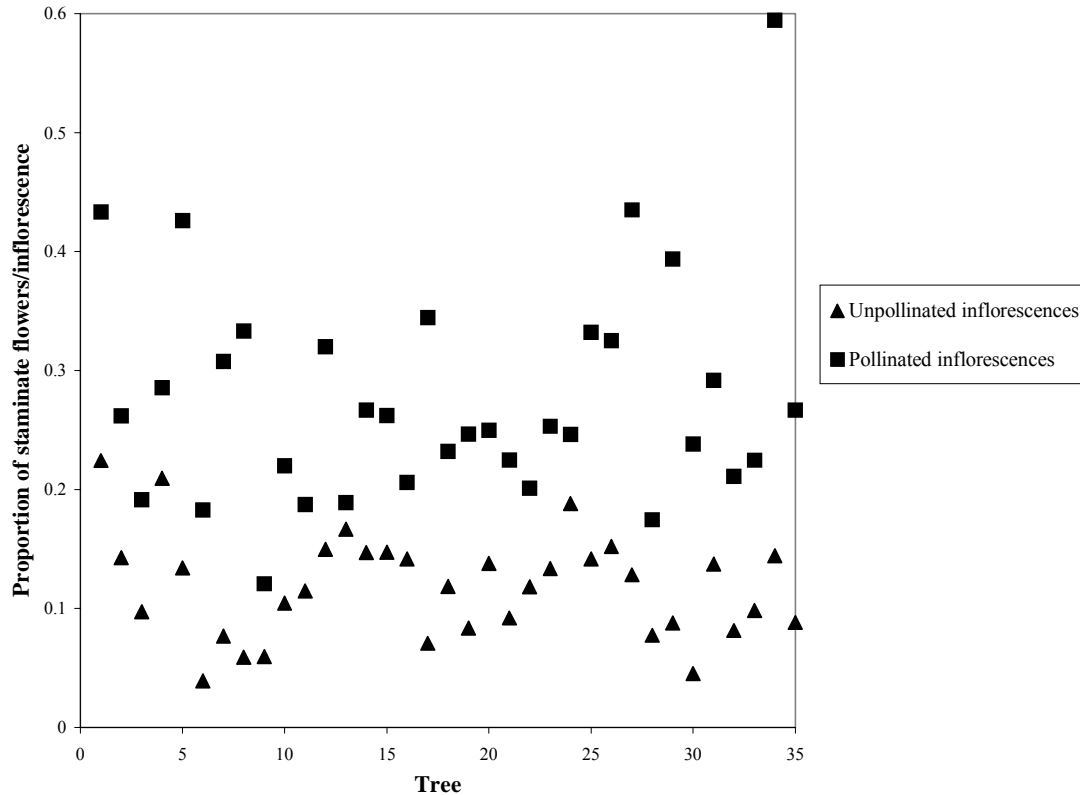
Figure 3.3b: Mean proportion of staminate flowers per inflorescence produced by 35 trees in pollinated (plus fruit) and unpollinated (no fruit) treatments during an investigation of inflorescence sex expression (Chamberlain, in press).

3.4   Breeding system

Breeding systems can be generally defined as those biological mechanisms that pass on the genetic material of one generation to another via sexual reproduction. Breeding systems therefore control genetic variability and are themselves controlled by the components of genetic variability (Richards, 1986). They regulate the distribution of genetic variation between the progeny of an individual, between individuals of a population and between populations or their sub-divisions (Hamrick, 1992). An understanding of the breeding system employed by a particular plant species can reveal degrees of inbreeding and the potential for hybridisation, as well as patterns of genetic structure. Tropical trees are thought to be predominantly outcrossing and self-incompatible, although a range of studies, often utilising molecular markers, have demonstrated the possibility that selfing still exists for some species (Moran and Brown, 1980; Murawski and Hamrick, 1991; Murawski *et al.*, 1990; 1994).

It had been assumed that *C. calothyrsus* was outcrossing and self-incompatible on the basis of: (1) its andromonoecious sexual system; (2) the slightly protandrous nature of the flowers; (3) the 10 mm difference in length between the style and stamens; and (4) its pollination by bats and large moths (Boland and Owour, 1996; Macqueen, 1992). A series of experiments involving controlled pollinations were carried out to confirm or reject this hypothesis.

Controlled pollinations

The breeding system of *C. calothyrsus* has been studied in a number of experiments using controlled pollinations. Rajaselvam *et al.* (1996) undertook controlled pollinations on three-year old trees from three provenances (Flores, Patulul and Turriabla) of *C. calothyrsus* established in a trial plot at Dodangolla, Sri Lanka. The fruit set on self-pollination was low (mean % fruit set = 2.7 (SE 2.7)), and crosses between provenances (mean % fruit set = 44.0 (SE 6.9)) were more successful than crosses within a provenance (mean % fruit set = 33.3 (SE 7.6)). Chamberlain (1996c) carried out controlled pollinations on three-year old trees from two provenances of *C. calothyrsus* (Flores and Santa María de Jesus) established in a trial plot in Cuyuta, Guatemala. In this experiment, the fruit set on self-pollination was also low (mean % fruit set = 4.4 (SE 0.9), but within provenance crosses were more successful (mean % fruit set = 26.0 (SE 3.0)) than between provenance crosses (mean % fruit set = 15.7 (SE 2.3)). The results of these two experiments suggest that incompatibility barriers between provenances of *C. calothyrsus* may be varied.

In a third experiment, undertaken in plot of *C. calothyrsus* established with trees from La Ceiba, Honduras, the fruit set resulting from within provenance crosses was similar to that found in the previous two experiments (mean % fruit set = 35.0 (SE 4.5) (Chamberlain, 1997). However, the fruit set on self-pollination was significantly greater on these nine-month old trees with a ten-fold difference in the number of fruits produced (mean % fruit set = 32.4 (SE 4.1)).

Controlled pollinations in *C. calothyrsus* have been conducted on different provenances grown on different sites and with trees of differing age. Variability in fruit set on selfing and cross-pollination has been demonstrated, and genetic variability in incompatibility barriers and tolerance of selfing between and within provenances could partly explain the results obtained.

Mechanism of self-incompatibility

Microscope observations of *C. calothyrsus* pistils have shown that pollen grains germinate readily on the stigma following both cross- and self-pollination (Chamberlain, 1997). After 18-21 h, pollen tubes resulting from both cross- and self-pollination reach the base of the style and can be observed in the ovary. Self-pollen was found to penetrate the ovules in the ovary, but with reduced success when compared to cross pollen (Rajaselvam, 1997). Sixty hours after pollination had taken place, 6.8% of pistils examined had penetrated ovules resulting from self-pollination, compared to 21.4% resulting from cross-pollination. No ovary enlargement of the selfed pistils had occurred at this time, which suggested that self-incompatibility in *C. calothyrsus* is gametophytic and may be late-acting. Observations of embryo development after self-fertilisation are needed to confirm whether the mechanism of rejection of self-pollen tubes is late-acting self-incompatibility, or a post-fertilisation rejection event (Gibbs and Bianchi, 1993).

Calliandra calothyrsus appears to be predominantly outcrossing, with a weak, gametophytic, possibly late-acting, self-incompatibility system. Provenance incompatibility barriers may be varied, and there may be a greater tolerance of selfing by some provenances. In natural populations, a variety of factors, e.g., floral phenology, population size and pollinator behaviour, may influence the outcrossing versus selfing rate, which may in turn affect the genetic structure of populations.

species (Chamberlain, 1996b; Hernández, 1991; Macqueen, 1992). The protuberance of the style beyond the anthers, and their combined distance from the corolla and nectary of an individual flower, suggested a large insect or mammal was responsible for pollination. Small insects, e.g., bees, wasps and beetles, have been observed to visit *C. calothyrsus*, but can only be regarded as 'nectar robbers' as these insects forage at the base of the stamens and corolla, gaining access to the nectar without coming in contact with either the anthers or the stigma. Rajaselvam *et al.* (1996) constructed cages around individual inflorescences that would permit the access of small insects and moths to the flowers of *C. calothyrsus*, but would exclude any large moths, birds or mammals. Less than 1% of the inflorescences treated in this way produced pods. Furthermore, the phenomenon of leaf folding at night (exposing inflorescences to potential pollinators), the 'brush' type flowers and the faint musky scent of the nectar (typical of bat-pollinated plants, and often more pronounced in other species of *Calliandra*) suggested bats and large moths were the primary pollinators of *C. calothyrsus*. Macqueen (1992) observed bats visiting the flowers of *C. calothyrsus* by either alighting, or hovering close to them. Bats removed the nectar with their long tongues, and pollen was smeared on their undersides. Macqueen suggested that the principal pollinator of *C. calothyrsus* in the native range was a small species of nectar-feeding bat, *Glossophaga soricina*. Hernández (1991) also observed floral visitors to *C. calothyrsus* in Mexico. He observed both bats and sphingid moths (*Eumorpha vitis* and *Erinnyis ello*) visiting flowers after 19:00 h.

More detailed observations on the pollinators of *C. calothyrsus* and their foraging behaviour were carried out through capture, mark and recapture experiments within four separate sites in the River Bonito, Colón, Honduras (Chamberlain, 1996b; Chamberlain and Rajaselvam, 1996). The sites were separated from one another by a minimum distance of 570 m, and a maximum distance of 3600 m. As suggested by Macqueen (1992), the most common pollinators of *C. calothyrsus* in this area were *Glossophaga* spp. (Glossophaginae), commonly known as nectar-feeding or long-tongued bats. They accounted for 79% of the captured bats and all of those recaptured (Figure 3.4). These small bats are characterised by the possession of long, narrow, extensible tongues covered in hair-like papillae that are specialised for nectar-feeding (Emmons, 1990). The remainder of the bats species captured belonged to the genera *Phyllostomus*, *Carollia*, *Sturnia*, *Artibeus* and *Uroderma*, all primarily fruit-eating species, but which will also consume pollen, nectar and insects to varying degrees (Nowak, 1994). Moths were not observed to be major pollinators of *C. calothyrsus* at this site. The recaptured bats were either caught at the site of original capture

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The breeding system of *C. calothyrsus* and its implications for seed production

♦ *Calliandra calothyrsus* is predominantly outcrossing, with a weak, gametophytic, possibly late-acting, self-incompatibility system.

♦ Provenance incompatibility barriers may be varied, with some provenances favouring within-provenance crosses rather than between-provenance crosses.

♦ There may be variability for tolerance of selfing in *C. calothyrsus*.

Implications: There is evidence that *C. calothyrsus* can tolerate selfing, the degree of which may be influenced by provenance origin, age, floral phenology, population size and pollinator behaviour. Outcrossing within *ex-situ* conservation stands and seed production areas can be promoted by ensuring that the seed used for establishment is genetically diverse, and that related individuals are not planted adjacent to one another.

3.5   Pollination system

The mode of pollination is an important factor in the reproductive biology of a plant species. Observations of pollinators and their movement are often difficult, however, and the relationship between pollinator movement and actual pollen transport may be confounded by the phenomenon of 'carry-over', in which pollen may be transferred across several plants before final deposition on the stigma (Levin, 1978; Schaal, 1980). This problem can often be overcome by applying molecular analyses to mating system models (see Chapter 7). The lack of suitable pollinators may be a factor causing flower abortion and low fruit set in plants (Bierzychudek, 1981; Koptur, 1984; Rathcke, 1983; Willson and Schemske, 1980). This may result either from a shortage of pollinators, or the inadequate transfer of compatible pollen by pollinators.

Floral morphology and phenology often provides the first indication of the pollination vector for a particular plant species. In *C. calothyrsus*, anthesis during the late afternoon and floral receptivity and nectar production during the early evening and night, suggested a nocturnal visitor was responsible for pollination in the

(later that evening [41.5%], or on another occasion [48.8%]), or between the two capture sites separated by the smallest distance of 570 m (9.7%). *Glossophaga* spp. have been found to possess restricted foraging ranges in other studies (360 m - Fleming *et al*., 1972; 380 m - Heithaus *et al*., 1975) relative to other, fruit-eating species, e.g., the spear-nosed fruit bat, *Phyllostomus discolor*, was found to forage over distances of up to 1600m (Heithaus *et al*., 1975). In this study, however, no data on the foraging distances of bat species other than *Glossophaga* was obtained due to low capture rates of the other bat species.

Primary pollination by nectivorous bats could have important implications for gene flow and population structure in the native range. The capture mark and recapture experiments described by Chamberlain and Rajaselvam (1996) suggest that pollen flow in natural populations could be more restricted than previously thought (Macqueen, 1992). This may have contributed to the high population differentiation observed among provenances of *C. calothyrsus* (Chamberlain, 1998a; see Chapter 7).
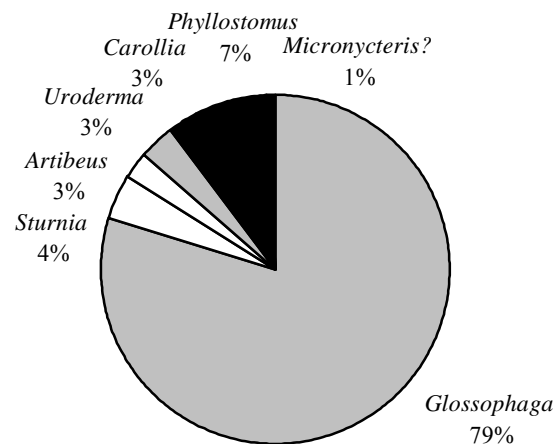
Figure 3.4:   Proportion of neotropical bat genera caught visiting *C. calothyrsus* in the River Bonito, Colón, Honduras.

The pollination system of *C. calothyrsus* and its implications for seed production

♦ Pollination in *C. calothyrsus* is effected predominantly by nectivorous bats that possess long, extensible tongues covered in bristle-like papillae.

♦ In the native range, these bats are members of the subfamily Glossophaginae, e.g. *Glossophaga soricina*.

♦ Less specialised fruit bats and large moths will also visit and pollinate *C. calothyrsus*.

♦ The nectivorous bats often forage over small distances, but longer-range pollination, either by these bats, or by fruit bats, cannot be discounted.

Implications: A suitable pollinator must be present in the vicinity of a seed production orchard. Seed production areas should be isolated from any other contaminating sources of *Calliandra* pollen in order to maintain the genetic identity of the seed produced. The minimum isolation distance will be dependent on the foraging ranges of the local pollinators but, as a guide, should be not less than 2 km. Bats may 'trapline'; hence, the establishment of trees in widely spaced rows may improve seed production.

3.6   Seed dispersal

The seed dispersal mechanism of *C. calothyrsus* is that of explosive apical dehiscence generated by drying tensions in the pod walls (Macqueen, 1992). The flattened pods, with thickened and raised margins, split from the apex to the base propelling seeds up to 10 m from the maternal tree. Seed is released from the funicle during dehiscence with a spinning motion, but can also remain in the open pod, dropping to the foot of the parent tree at a later date. In general, therefore, the pollination and seed dispersal potential of *C. calothyrsus* in many natural populations may be restricted.

Where *C. calothyrsus* occurs as a coloniser of riverbanks and streams, water can take the seed downstream. In such riverine populations, the potential exists for long range seed distribution. Results from the isozyme analysis of sub-divided populations (Chamberlain, 1997) lends support to this view. Populations found in river systems, and in which individuals also colonise nearby roadsides and fallow fields, e.g., at La Ceiba and Bonito Oriental, Honduras, and at Fortuna, Costa Rica, extend over distances of up to 2-12 km. The individuals within these populations show a high degree of similarity and little variation between sub-populations.

This has important implications for seed collection strategies and suggests that seed should only be collected from trees at least 100 m apart to reduce consanguinity. Sampling more than one individual from a small clump of *C. calothyrsus* should be avoided, and instead the population as a whole should be widely sampled to maximise the genetic variation in the seed collected.

3.7   Inter-specific hybridisation

Species within *Calliandra* series *Racemosae* typically display allopatric distributions, with geographic isolation rather than reproductive isolation, suggested as the reason for speciation within this group of taxa (Macqueen and Hernández, 1997). Occasionally, sympatric populations (different species occupying the same geographic area) have been observed, which may have arisen as a result of habitat disturbance, followed by colonisation by one or other of the species. Sympatry primarily exists between *C. calothyrsus* and *C. houstoniana, C. houstoniana* and *C. grandiflora*, and *C. houstoniana* and *C. juzepczukii*, and putative hybrids have been documented between *C. calothyrsus* and *C. houstoniana*, and *C. houstoniana* and *C. juzepczukii* (Chamberlain, 1996c; Macqueen and Hernández, 1997). Although, inter-specific hybridisation has the potential to provide interesting new combinations of characters, only

recently have these putative hybrids, and the mechanisms by which they may have arisen, been investigated.

Natural hybridisation

Interspecific hybridisation between *Calliandra* series *Racemosae* was suspected to occur naturally between *C. calothyrsus* and *C. houstoniana* in Honduras (near Meambar, Comayagua and Santa Rosa, Copán) and in Mexico (Colotlipa, Guerrero and Yajalón, Chiapas), and between *C. houstoniana* and *C. juzepczukii* in Mexico (Ocozocautla, Chiapas) (Macqueen and Hernández, 1997). If speciation has occurred in the past through a process of geographic rather than reproductive isolation, habitat disturbance following human activity (e.g., agriculture and road construction) in combination with the pioneer nature of many *Calliandra* species, may have led to hybridisation between previously separated species. Hybridisation is generally associated with the loss of valuable provenances and taxonomic confusion. Such concerns have been highlighted for *Gliricidia sepium,* which has been shown to hybridise with *G. maculata,* raising the possibility that the genetic integrity of the important provenances on the Pacific coast of Guatemala could be threatened (Dawson *et al.*, 1996).

Field studies at five sites where sympatric populations of *C. calothyrsus* and *C. houstoniana* occur (Copán, Honduras; Nuevo Ocotepeque, Honduras; Meambar, Honduras; Chicosan, Mexico; Flores, Guatemala) were undertaken by Chamberlain (1996c). At each site, with the exception of Flores, no putative hybrids could be morphologically identified, despite an overlap in the individual species' flowering times. The two species were found generally to occur in small, separate 'micro-environments' that could favour one species over another and would select against any hybrids. For example, in Chicosan, Mexico, *C. houstoniana* was found in seasonally dry deciduous forest and *C. calothyrsus* found by a wet cliff face and in a nearby riverbank 500m from the nearest *C. houstoniana* (Chamberlain, 1997). In Flores, however, trees of intermediate morphology were found in areas containing *C. houstoniana* subsp. *houstoniana, C. calothyrsus* and *C. houstoniana* subsp. *stylesii* where all three species were intimately mixed. These intermediate forms also appeared to be largely infertile; for example, one specimen produced flower buds without their floral and reproductive organs. In general, pod production also appeared to be low on the intermediate types compared to the neighbouring true species. The intermediates appeared to have similar vegetative characters as *C. calothyrsus* but with the harder, waxy leaves of *C. houstoniana*. In addition, the stigmas of the intermediates were capitate, as in *C. houstoniana,* rather than cup-shaped as in *C. calothyrsus*. The presence of *C. houstoniana* subsp.

stylesii at Flores was also interesting because it displays morphological characteristics intermediate between *C. houstoniana* subsp. *houstoniana* and *C. calothyrsus,* and may itself be of hybrid origin (Macqueen and Hernández, 1997).

Vegetative and reproductive samples of *C. calothyrsus*, *C. houstoniana* subsp. *houstoniana*, the intermediate form and *C. houstoniana* subsp. *stylesii* were collected from two locations near Flores and analysed using morphometric techniques (Chamberlain, 1996c; Hubert, 1997). The leaf material was also used for molecular analysis, and the results of this work are discussed in Chapter 7 (Hubert, 1997). The results of the morphometric analysis strongly suggested that the intermediate form of *Calliandra* was of hybrid origin with all the morphological characters tested being intermediate between the two parents, *C. calothyrsus* and *C. houstoniana* subsp. *houstoniana*. This 'intermediacy' can be visualised by plotting the first two principal components (Figure 3.5).

Results of the analysis were not as clear for *C. houstoniana* subsp. *stylesii*. One part of the analysis provided evidence for a divergent origin of the taxon, i.e., segregation and differentiation within a parent taxon (the most likely being *C. houstoniana*) to the extent that *C. houstoniana* subsp. s*tylesii* was derived. The second part of the analysis suggested a possible hybrid origin of *C. houstoniana* subsp. *stylesii*, but with *C. houstoniana* having had a dominant effect on its evolution.

Overall, the intermediate form of *Calliandra* observed around Flores, Guatemala, was almost certainly of hybrid origin, but the status of *C. houstoniana* subsp. *stylesii* is not clear and it is difficult to state whether the species is of hybrid or divergent origin. If of hybrid origin, its greater similarity to *C. houstoniana* subsp. *houstoniana* may have been due to the production of a fertile hybrid that then backcrossed with *C. houstoniana* subsp. *houstoniana* to produce the species observable today. Further research is needed to fully elucidate the evolution of *C. houstoniana* subsp. *stylesii*.
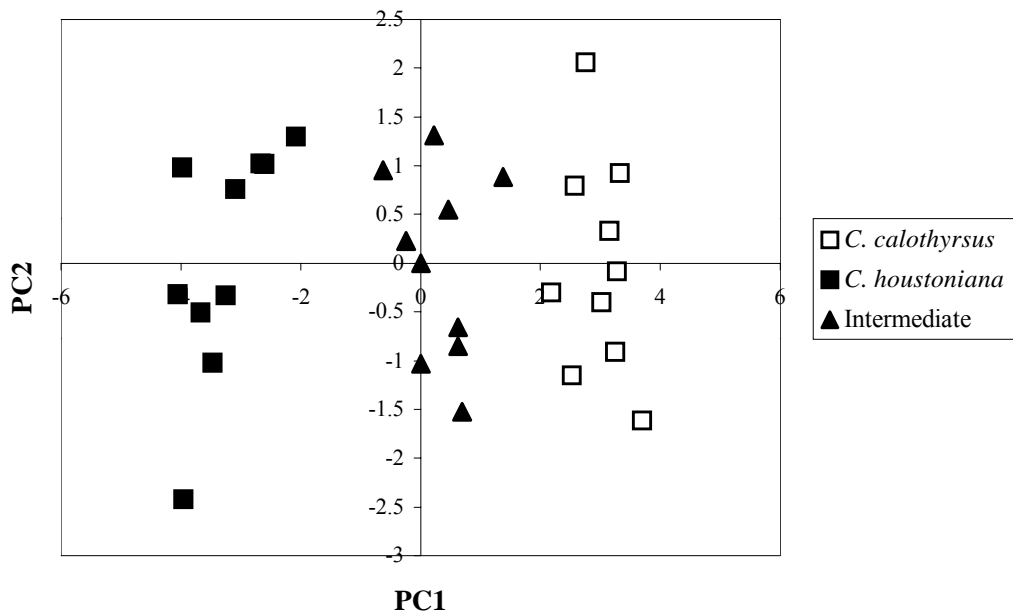
Figure 3.5:   PCA plot of the first two principal components for morphometric traits of *C. calothyrsus*, *C. houstoniana* subsp. *houstoniana* and the putative hybrid (Intermediate) collected from Flores, Guatemala.

Artificial hybridisation

Controlled pollinations were carried out between three species of *Calliandra* planted in a trial in Honduras (Chamberlain, 1997). Reciprocal crosses were made between flowers of *C. calothyrsus, C. houstoniana* subsp. *houstoniana*, and *C. juzpeczukii*. All inter-specific crosses were successful with the exception of those between *C. calothyrsus* and *C. juzpeczukii*.

Percentage fruit set was generally greater in the intra-specific crosses, but viable seed was produced in all cases. For each species, there were significant differences between pollination treatments for percentage fruit set and mean seed ovule ratios. The mean percentage fruit set for the reciprocal crosses involving *C. calothyrsus* and *C. houstoniana* were significantly different from one another (4.4% as opposed to 18.6%), perhaps indicating that *C. calothyrsus* has a stronger inter-specific incompatibility mechanism than *C. houstoniana*. For each of the species, fruit set after selfing was low.

Seed resulting from the above experiment was planted as a trial in Honduras to monitor the growth and reproductive success of the hybrids (Chamberlain, 1997). Early results have indicated that the fastest growing hybrid, in terms of plant height, was that between *C. houstoniana* (♀) and *C. calothyrsus* (♂). This hybrid was significantly taller than its reciprocal cross, *C. calothyrsus* (♀) x *C. houstoniana* (♂). These results were obtained after only six months growth, hence any conclusion of hybrid vigour remains tentative. The role of *C. houstoniana* as the maternal parent, both in terms of the growth of the hybrid and the species' inter-specific incompatibility, merits further investigation. The production of inflorescences was also monitored in the trial, and structurally perfect flowers were produced by all the hybrids after six months. Neither the *C. houstoniana* or *C. juzpeczukii* controls had flowered at this stage, therefore, precocious floral development may be characteristic of their hybrids.

Inter-specific hybridisation is clearly a feature of *Calliandra* series *Racemosae*, and the barriers to cross-fertilisation between *C. calothyrsus* and *C. houstoniana,* and *C. houstoniana* and *C. juzpeczukii,* are few. Natural hybrids appear to be uncommon, however, and are likely to have arisen through the disturbance of previously isolated habitats. When species come together in sympatric populations, hybridisation does not always occur, as localised sub-populations of each species often appear to be associated with a particular ecological niche. In this situation, the relatively short ranges over which pollinators may forage for nectar may also favour intra-specific crosses (see section 3.5). Where hybridisation is possible, there are important implications for the genetic identity of seed collected from such sites. Seed should be collected from large stands of the desired species that are isolated from any other *Calliandra* species. Seed should not be collected from stands where trees from more than one species are intimately mixed within a few metres of one another.

Hybrids between *C. calothyrsus* and *C. houstoniana* may have some potential within improvement programmes. Clearly hybrids can be produced, and although natural hybrids appear to be largely infertile, the presence of structurally perfect flowers on the artificially produced hybrids indicates that further research is required to fully investigate the fertility of *Calliandra* hybrids. Similarly, the artificial hybrids appear to show some vigour in terms of growth, but this needs to be evaluated further before the growth characteristics of the hybrids can be determined clearly.

Inter-specific hybridisation in *Calliandra* and its implications for genetic improvement

Natural hybridisation

♦ Although sympatric populations of species within *Calliandra* series *Racemosae* have been observed, putative hybrids are rarely found.

♦ An intermediate morphological form observed in Flores, Guatemala, has been identified as a hybrid between *C. calothyrsus* and *C. houstoniana* subsp. *houstoniana*, and exhibited reduced fertility.

Implications: With the increasing disturbance of natural forest habitats, the incidence of inter-specific hybridisation in *Calliandra* may increase. This has implications for the genetic integrity of seed collected in areas with sympatric populations of *Calliandra* spp. Seed should always be collected from large stands of the desired species that are isolated from other *Calliandra* species. Seed should not be collected from stands where more than one species are intimately mixed.

Artificial hybridisation

♦ Artificial hybrids have been produced between *C. calothyrsus* and *C. houstoniana* and between *C. houstoniana* and *C. juzepczukii.*

♦ Preliminary results from a trial in Honduras suggest that there may be hybrid vigour for growth.

♦ Precocious flowering may be associated with hybrids, which produced structurally perfect flowers.

Implications: The results from the study of inter-specific hybridisation in *Calliandra* are at a preliminary stage, hence it is difficult to draw definite conclusions. With this in mind, hybrid vigour for growth characteristics may exist, with crosses between *C. houstoniana* (♀) and *C. calothyrsus* (♂) being the most productive. The fertility of such a hybrid is open to question given the conflicting observations between natural and artificially produced hybrids.

3.8   Reproductive biology and seed production

As discussed at the beginning of this chapter, seed production in *C. calothyrsus* can be limited, both in the native range where small populations composed of scattered individuals are found, and in exotic environments where unsuitable climatic conditions, or a lack of pollinators may restrict the quantity of seed produced by the species. Studies on the reproductive biology of *C. calothyrsus* have provided valuable information relating to the mechanisms that control seed production in the species. Recommendations have therefore been made on the design and management of seed production areas with the aim of maximising the quantity of seed produced (Chamberlain, 1996b; 2000a). Similar considerations can apply when establishing *ex situ* stands of *C. calothyrsus* for conservation purposes (see Chapter 8).

Studies on sex expression in *C. calothyrsus* have suggested that an endogenous limit may be placed on the number of hermaphrodite flowers an individual tree in a particular environment can produce. Trees can respond to soil fertilisation by increasing the number flowers produced and increased seed production can follow. A seed orchard of *Gliricidia sepium* planted in Indonesia responded well to fertiliser application, which increased the proportion of trees flowering from 44 % to 71 % (Simons, 1996). In a seed production orchard of *C. calothyrsus* in Australia, the trees were both irrigated and fertilised, and the resulting seed yields have been very good (J. Hopkinson, pers. comm.[3]). Although soil fertilisation within seed production areas and its effects need to be tested experimentally, the experience in Australia and general responses to soil fertilisation by other plants suggest that *C. calothyrsus* may respond favourably through increased numbers of hermaphrodite flowers, and increased seed production.

Studies on the breeding system of *C. calothyrsus* have shown the species to be predominantly outcrossing, with a weak, gametophytic, possibly late-acting, self-incompatibility system. In order to maximise the number of compatible crosses effected by pollinators, and avoid selfing and the associated risk of inbreeding, a genetically diverse seed source should be used to establish seed production areas. As well as promoting seed yields, this strategy will also ensure that diversity is maintained in the resultant seed crop.

Suitable climatic conditions and the presence of pollinators are, however, the key to ensuring good seed yields are obtained in seed production areas of *C. calothyrsus*. Although little information is available on

the climatic conditions most suited to *C. calothyrsus* seed production, some general guidelines can be deduced from the species' habitat in the native range. The species occurs across a wide range of habitats, from sea level to 1850 m above sea level. Mean annual rainfall at these sites varies from 700 mm to over 4000 mm. Poor seed yields, that may in part be attributable to climatic factors, have been obtained in higher altitude areas, which experience cool persistent mists at night, or where rainfall is at the highest end of the range (Macqueen, 1993). Such conditions are likely to interfere with pollen dehiscence and hence, the effective transfer of pollen by pollinators. Areas with similar conditions should be avoided when siting seed production orchards.

Knowledge about the pollinators of *C. calothyrsus*, and their feeding and flight patterns, can have a profound effect on the quantity of seed produced. Nocturnal flowering and the distance of the reproductive parts (anthers and stigma) from the nectary means that only bats and large moths are routinely effective pollinators of the species.

The type of pollinator and its behaviour has special implications for *C. calothyrsus* when the species is cultivated in exotic environments. From observations in the native range, small bat species adapted for nectar-feeding are likely to be the most efficient pollinators. Amongst the Old World fruit bats (Pteropodidae), the members of the subfamily Macroglossinae are characterised by the possession of a long, extensible tongue covered in bristle-like papillae, much as in the Glossophaginae (Mickleburgh *et al*., 1992). These bats are native to west and central Africa, south-east Asia, the Pacific Islands and northern Australia. Macroglossinae bats are likely to be the most efficient pollinator of *C. calothyrsus* when it is planted in exotic environments, and were found to pollinate the species in Indonesia (Chamberlain and Rajaselvam, 1996). In areas where these bats are not native but where *C. calothyrsus* is cultivated, e.g., east Africa and Sri Lanka, other species of bats visit *C. calothyrsus*. In Sri Lanka, flowers of *C. calothyrsus* were visited by two genera of fruit bat, *Cynopterus* and *Rousettus* (Pteropodinae) (Chamberlain and Rajaselvam, 1996). These bats appear to visit *C. calothyrsus* flowers much less frequently than bats in the native range and alter their feeding habits according to seasonal food availability, but are effective pollinators when they visit. In Australia, *C. calothyrsus* was pollinated by flying foxes (*Pteropus* spp.) which have a very different means of feeding on the species' floral resources (Matthews and Hopkinson, 1998). Flying foxes land on the tree, scramble along the branches inside the tree, approach the inflorescences from the inside and collect nectar and pollen from the flowers. They have been observed to forage over a tree

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[3] Department of Primary Industries, Research Station, Walkamin, Queensland 4872, Australia.

in a matter of minutes, and may be very effective pollinators of *C. calothyrsus*.

It is possible that there may be a time delay before suitable pollinators recognise the new food resource a stand of *C. calothyrsus* represents. For instance, a seed production area planted in Honduras in 1993 was first visited by pollinators two months after the trees had begun flowering, despite the presence of bats and large moths visiting *Calliandra* flowers 1-2 km from this site. A similar example has been reported from Kenya (A.J. Simons, pers. comm.[4]). The quantity of *C. calothyrsus* flowers produced and the length of the flowering period will be critical in the competition for pollinators with other flowering or fruiting plant species. The larger the stand of *C. calothyrsus*, the more attractive the food resource may be to pollinators. An assessment of the floral and fructal phenology in other nearby species, i.e., potential competitors for pollinators, may also aid decisions on where to site a seed production area.

Any *C. calothyrsus ex-situ* conservation stand or seed production area should be isolated from other contaminating sources of *Calliandra* pollen in order to maintain the genetic identity of the seed produced. The minimum isolation distance will be dependent on the foraging ranges of the local pollinators. This could be quite restricted, as in the case of small, nectivorous bats, but larger, fruit-eating bats have the potential to forage across much greater distances. The movement patterns of bats between and within trees of *C. calothyrsus* may also be a factor in the design, spacing and management of a seed production area. For example, a seed production orchard in Australia has trees in wide rows at a spacing of 2 x 8 m (Matthews and Hopkinson, 1998). Seed orchards in Kenya have been established at a variety of spacings (2 x 2 m, 2.5 x 2.5 m and 3 x 3 m) in order to evaluate which is the most productive planting design (A.J. Simons, pers. comm.[4]). For the majority of pollinators, inflorescences that have prominent positions at the top or sides of the tree are more likely to be visited than those concealed from view by other branches on the same tree, or by adjacent vegetation. The ease of access to inflorescences is therefore likely to increase the chance of effective pollination, hence wide spacing in blocks, or line planting in wide rows are likely to produce the best results in terms of seed production per unit tree.

The seed dispersal mechanism of *C. calothyrsus* and the sequential ripening of its pods often make seed collection from orchards a difficult and time-consuming activity. One method of collection used in Australia that may prove useful elsewhere, is the use of hessian sacking stretched on the ground between rows of *C. calothyrsus* (Matthews and Hopkinson, 1998).

The seed is allowed to dehisce naturally and is swept from the hessian at regular intervals using a hand-held blower.

Recommendations for improved seed production in *C. calothyrsus*

♦ Establish a seed production area with seed from a single, genetically diverse provenance.

♦ Choose a site with suitable climatic conditions where nectivorous bats or moths are known to be native.

♦ Plant the orchard at a wide spacing, e.g., 3 m x 3 m, or in wide rows, e.g. 2 m x 8 m.

♦ Isolate the seed production area from any other sources of *Calliandra* pollen.

♦ Minimum isolation distance should be 2 km, but bats can forage over larger distances.

♦ Assess the likelihood of competition for pollinators by other plants, e.g., figs, banana.

♦ A large seed production area will represent an attractive food source and maintain genetic diversity in the resultant seed crop.

♦ Do not expect a good seed crop after the first flowering year.

♦ Prune annually to approximately 1 m prior to the main rainy season to promote the production of inflorescences.

♦ Fertilise the soil below the trees in the stand to promote the production of hermaphrodite flowers.
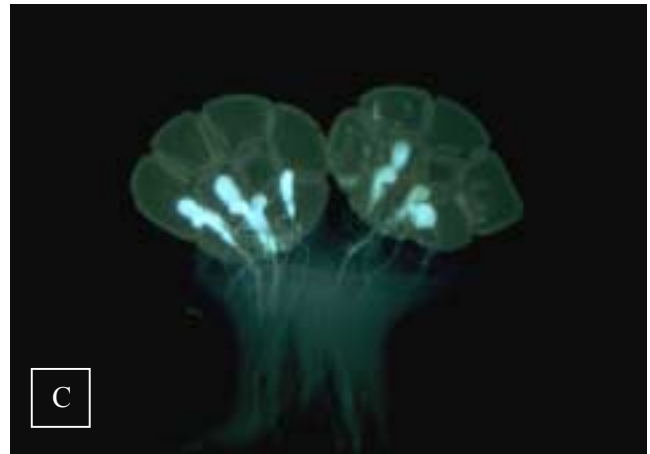
Plate 2

A - Buds of *Calliandra calothyrsus* bursting to expose the style and stamens.

B - An inflorescence of *Calliandra calothyrsus* showing emasculated flowers manipulated during experiments involving controlled pollinations.

C - Fluorescent microscopy revealing two polyads and their pollen tubes germinating on the stigma of *Calliandra calothyrsus*.

D - Three pollen tubes in the ovary of *Calliandra calothyrsus*.

E - *Glossophaga soricina*, one of the primary pollinators of *Calliandra calothyrsus* in the native range.

4   Seed collection and propagation

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4.1   Introduction

Until recently, the majority of *C. calothyrsus* seed has been collected in, and supplied by Indonesia where there are large, naturalised stands of the species and seed is abundant. Concern that these seed sources contain little genetic variation and may be vulnerable to pests or disease attack, led to the collection of *C. calothyrsus* seed from throughout its native range for evaluation and possible incorporation into agroforestry programmes (Macqueen, 1991). This chapter describes the strategies employed in the seed collection programme and discusses how seed of *C. calothyrsus* should be handled, stored and propagated. Methodologies for vegetative propagation of the species are described and the relative merits compared with propagation by seed discussed.

4.2   Seed collection

Seed collection for provenance evaluation

A range-wide collection of *C. calothyrsus* seed was initiated in 1990 by the Oxford Forestry Institute with the aim of sampling and evaluating genetic diversity in the species in order to provide farmers with well documented, productive and genetically diverse seed for use in a range of agroforestry systems (Macqueen, 1991). Provenances were delimited on the basis of the species' distribution across different environments in the native range (Macqueen, 1992). Seed was collected from as many of these provenances as possible. Sampling within provenances balanced the need to sample a large number of trees whilst maintaining genetic diversity and reducing the likelihood of relatedness. Macqueen (1992) recommended the collection of at least 50 individuals spaced at distances greater than 100 m, but noted that the size and density of populations often required a more pragmatic approach. Collections were, therefore, made from between 8-65 individual trees (most commonly between 20-50) spaced at a minimum of 50 m intervals (Macqueen, 1993a). The presence of root suckering in one population in northern Honduras meant that adjacent plants were genetically identical and highlighted the need to collect from well-spaced individuals. Seed was collected from trees chosen at random within a provenance to ensure that a wide spectrum of genotypes was sampled. No more than 75% of pods on a particular tree were collected in order to leave sufficient seed for the natural regeneration of the stand.

In the native range, flowering in *C. calothyrsus* is at a peak during October to January. The time from flowering to pod dehiscence is approximately 90-120 days (Boland and Owour, 1996). Seed set peaks between the months of January and April, but varies, being between January and February in the drier environments of Mexico, Guatemala, Honduras and Nicaragua, and between March and April in the more humid environments of Guatemala, Honduras, Costa Rica and Panama (Macqueen, 1992; Macqueen and Hernández, 1997). The explosive dehiscence of *C. calothyrsus* pods means that accurate timing for seed collection is essential. Ideally, collection should take place when fruit maturation is at a peak and the majority of trees within a population set seed. Flowering within *C. calothyrsus*, however, is sequential from the base of the inflorescence to its tip, and there will nearly always be a proportion of seed on a tree that is not ripe. If the population is small, or flowering and subsequent fruiting between trees has been very asynchronous, then repeated visits to a site at intervals of three weeks may be required to obtain sufficient quantities of seed. Different inflorescences can also set different quantities of seed, perhaps due to pollinator foraging behaviour and the ease of access pollinators have to an inflorescence. Nevertheless, seed should be collected from throughout the canopy to ensure that the variation in pollen contributions from other trees is sampled (Chamberlain and Rajaselvam, 1996).

Seed-ovule ratios per pod have been found to vary both within- and between-populations of *C. calothyrsus*. Measurements from a number of populations within the native range found mean seed ovule ratios to vary from 0.83 to 0.65 per pod per population (Chamberlain, unpublished results; Hernández, 1991; Macqueen, 1993b). Mean ovule number varied from 9.1 to 6.7, and mean seed number per pod varied from 6.6 to 5.5. In the native range, quantities of seed per tree for *C. calothyrsus* are low (averaging about 100 g per tree, equivalent to approximately 1,400-1,700 seeds: Macqueen, 1993a; NRC, 1983). Hence, small quantities of seed per tree should not always be interpreted as a seed production problem when the species is introduced to exotic sites (Chamberlain and Rajaselvam, 1996; Boland and Owour, 1996).

Seed collection from the native range of *C. calothyrsus* allowed more than 70 provenances to be assembled and used for research and evaluation purposes.

Seed collection for tree planting on farms

For the practical use of *C. calothyrsus*, seed will be collected from trees planted on farmland, or from an area planted specifically for seed production. In such situations, it is useful to gather as much information as possible about the stand from which seed is to be collected, especially regarding the ancestry of the trees. Stands established with seed from a small number of trees might be of low genetic diversity and their progeny vulnerable to pest and disease attack.

It is good practice to collect seed from *C. calothyrsus* when it is fully mature, i.e., just prior to natural dehiscence, since unripe seeds will have low viability (Willan, 1985). The sequential ripening of pods along the axis of the inflorescence means that, at any one time, only a proportion of the seed available will be ripe, and the separation of mature and immature pods at this stage is important if high germination figures are to be achieved later on. Pods change colour from green to golden or dark brown as seed reaches maturity. Ripe seeds are hard and dark-brown in colour. In addition, seed should always be collected during dry conditions to minimise the potential for rotting and pest or disease attack.

Long-arm pruners may be used to cut inflorescence axes bearing mature fruit, or the branches can be bent down and the pods removed by hand. In seed production orchards, sacking or nets have been placed below the trees. Pods are then allowed to dehisce naturally, and the majority of a tree's seed crop can be collected (Matthews and Hopkinson, 1998). This latter method is not usually practicable where trees occur in farmers' fields, or in dense vegetation.

Seed predation by bruchids, e.g., *Stator limbatus* (Johnson and Lewis, 1993), varies between sites from virtually nil to 85% of the total seed harvest (Macqueen, 1993b). Once seed is separated, cleaned and placed in canvas bags for interim storage, regular checks can be made to remove adult bruchids as they emerge from the seed. Low temperature storage of seed is an effective way of killing any further developing beetles.

4.3   Seed handling and storage

Reducing the time between seed collection and storage is critical to maintaining high viability and good

germination rates (Bonner *et al.*, 1994). In addition, every effort should be made to minimize damage during cleaning, extraction, drying and storage so as to maximize viability (Poulsen, 1993). For both native and exotic stands of *C. calothyrsus*, the collection season is usually correlated with the beginning of the dry season. It is therefore often possible to use sun-drying, both of pods and seeds. The separation of seeds from pods is best achieved through slow natural drying. Pods may be placed on wire mesh beds and left in the sun to open. The wire mesh traps the seed as the pods dehisce. Where possible it is best to avoid manual opening of the pods as seed from such pods may undergo overly rapid and potentially deleterious drying on direct exposure to the sunlight. When sun-drying is not possible, pods can be placed in ventilated sacks or wire mesh cages above a current of warm air provided by an electric drier or gas stove. It is vital that pods are placed sufficiently high above any direct heat to avoid excessive temperatures and overly rapid drying.

Once pods are open, seed can be threshed out from the pod waste and cleaned manually, or by any appropriate mechanical cleaning device. At this stage it is important to dry the seed to between 6-10% moisture content by placing it on open, drying mats, preferably avoiding prolonged exposure to direct sunlight where temperatures may rise unacceptably and reduce moisture content to below the ideal. In wet conditions, seed may be dried using an electric hot air blower, by placing the seed high above a stream of hot air from a stove, or by placing it in bags with silica gel. Dry seed should be stored in clearly labelled ventilated canvas bags (since plastic bags hold residual water and may allow infestation with fungi).

Seed moisture content and temperature are critical to successful long-term storage. Bonner *et al.* (1994) note that orthodox seeds, such as those of *C. calothyrsus*, can tolerate very low moisture contents of less than 10%. In addition, they can be stored at temperatures below zero with greatly extended viability provided the moisture content is sufficiently low prior to freezing. C.M. Surman[1] (unpublished data) demonstrated that in general there is no significant difference in germination percentage between seed of *C. calothyrsus* stored at room temperature (25ºC) and 4ºC during the first 12 months of storage. For storage periods of up to five years, seed can be stored at 4°C with germination percentages of 75-90%. Ideal storage containers are air-tight metal tins which protect the seed from external changes in humidity and prevent insect and fungal attack. However, problems have sometimes been reported with seed viability (e.g., Roshetko *et al.*, 1996) and the need to reduce the time between collection in the field and storage under cool, dry

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conditions should be emphasised.

When small quantities of seed are collected from farmland for direct sowing, low temperature storage facilities are usually not available. Under such conditions, seed should be dried according to the regime described above, and stored in a cool and dry location. The seed should be planted as soon as possible after collection, rather than kept for prolonged periods.

4.4   Propagation and establishment

In common with a number of other agroforestry trees, *C. calothyrsus* does not propagate readily from stakes, and although vegetative propagation is possible, by far the most common means of propagation is from seed. Seed can either be sown directly at the planting site, or raised in the nursery as container seedlings. Selection of the appropriate propagation technique will depend on the planting environment (soil fertility, rainfall, etc.), the availability of seed, financial resources, and labour and transport constraints. Standard propagation techniques have been widely reported elsewhere (e.g., Rojas, 1987; Weber, 1977), so the information supplied here has been restricted to that directly relevant to the propagation of *C. calothyrsus.*

Seed pre-treatment

Seed of *C. calothyrsus* can germinate without any pre-treatment, particularly when fresh seed is used. Nevertheless, improved germination can be achieved either by making an incision in the seed coat, or by brief soaking in hot water followed by overnight soaking in cold water. Verhoef (1939) reported an increase in germination rate from between 28-48% (untreated) to 94-97% (with nicking). Halliday and Nakao (1984) also found scarification to improve germination. Verhoef (1939) found that the treatment of seed with boiling water did not affect the germination rate, but soaking for five minutes in concentrated sulphuric acid lowered the germination rate to 53%. Diangana (1985) similarly found that three minutes hot water treatment at 80°C had no negative effect on germination, and that soaking in cold water for 24 hours gave better results than 30 minutes in hydrogen peroxide, or one hour in dilute sulphuric acid. Such results appear to contradict reports that hot water treatment hastens germination (Lowry and Macklin, 1988; Satjapradja and Sukandi, 1981). CATIE (1986) have recommended a pre-treatment of 10 minutes in water at 70°C followed by 24 hours in cold water. This treatment was similarly endorsed by Wiersum and Rika (1992), and in situations where labour constraints make individual nicking of seed unjustifiable, 10 minutes in hot water followed by 12-24 hours soaking in cold water may be the preferred option.

Inoculation

Calliandra calothyrsus fixes atmospheric nitrogen through a symbiotic relationship with strains of *Rhizobia* bacteria contained in root nodules. Nodulation occurs most rapidly between temperatures of 28-32°C (Purwantari *et al.*, 1995). The growth and shoot nitrogen content of the species is greatly improved by inoculation with *Rhizobium* in comparison with uninoculated controls (Lesueur *et al.*, 1996a). *C. calothyrsus* was scarcely, or not at all, nodulated by strains from the genus *Bradyrhizobium,* but up to 100% of experimental plants were nodulated by specific strains of *Rhizobium* (Lesueur *et al.,* 1996a). Purwantari *et al.* (1996) found that *C. calothyrsus* nodulated with both *Rhizobium* and *Bradyrhizobium*, but that *Rhizobium* gave the largest nodule dry mass and N fixation. Other researchers have also found preferential nodulation with *Rhizobium* (Bekunda, 1993; Turk and Keyser, 1992), suggesting that while *C. calothyrsus* may be promiscuous in its strain requirement, it most readily nodulates with the appropriate *Rhizobium*, but not *Bradyrhizobium.*

Calliandra calothyrsus belongs to a group of woody legumes that are specific both for nodulation and the effectiveness of that nodulation (Lesueur *et al.*, 1996a; Odee, 1989). While nodulation in exotic environments may occur with indigenous strains of *Rhizobia,* such nodulation may, or may not, be effective in fixing nitrogen (Halliday and Somasegaran, 1982). In view of this, it has been recommended that when introducing *C. calothyrsus* to a particular exotic location for the first time, inoculation with effective, known strains of *Rhizobium* should be undertaken. Macqueen (1993a), in conjunction with NifTAL, recommended and supplied the strains TAL 583, TAL 1145, TAL 1770, TAL 1806, and TAL 1887 for use in the international *Calliandra* trial network. Lesueur *et al.* (1996a; 1996b), however, identified eight strains that gave better results than three comparable NifTAL strains (CCR10, CCR13, CCR17 and CCR20A from Reunion, and CCK3, CCK6, CCK10 and CCK13 from Kenya), although the strains were not tested under field conditions. Lesueur *et al.* (1996a) found that different strains were diverse in terms of their physiological and biochemical properties, which may have implications for their effectiveness when *C. calothyrsus* is grown on different sites.

Rhizobium inoculum should be stored in a sterile, dry peat medium. It should be kept sealed and refrigerated in a dark environment and used within six months of preparation. Inoculum can be applied as a coating to

seed using 50 g of inoculum per kg of seed. Alternatively, a slurry of 5 g inoculum mixed with water can be applied directly to 1000 seedlings.

Another potentially important soil association is that with vesicular-arbuscular mycorrhizal (VAM) fungi. Reena and Bagyaraj (1990) found that when *C. calothyrsus* was inoculated with *Glomus velum* and *Glomus merredum* (VAM fungi), plants had greater height, leaf number, stem girth, biomass, and phosphorous and zinc content than uninoculated plants. In a pot experiment, Ibrahim *et al.* (1996) noted a similarly positive effect of VAM inoculation on plant height, stem diameter and root biomass, provided there was adequate phosphorous in the soil. The increase in above ground biomass of VAM-infected *C. calothyrsus* with phosphorous application was proportional to a supply of between 16 and 64 kg/ha phosphorous. It was therefore suggested that VAM infection may mediate the response to phosphorous application.

Care must be taken when preparing land for *C. calothyrsus* cultivation to minimise the duration of the preceding clean cultivation (i.e., plants without rhizobial and mycorrhizal associations) which may reduce the land's inoculation potential (Shepherd *et al.*, 1996). Where this is unavoidable, direct inoculation may provide benefits. Current research that aims to evaluate strains of both VAM fungi and *Rhizobium* from the species' native range should further improve the inoculum which can be supplied with germplasm of *C. calothyrsus* (D. Lesueur, pers. comm[2].).

Direct sowing

Direct sowing of *C. calothyrsus* in the field can be successful, but generally shows low survival rates. In Indonesia, Soerjono and Suhaendi (1981) observed that with seed sown by hand, five seed are placed in a single hole at the required planting spacing. Where seed is abundant (e.g., in Java) direct sowing has proved attractive, with up to 60-70% survival rates per sowing hole (Hadipoernomo, 1979). In Jamaica, however, Roshetko *et al.* (1996) reported low survival rates (only 11%) from direct sowing, where a subsequent dry period and competition from pasture grasses led to high levels of plant mortality. While direct sowing greatly reduces labour inputs in comparison to the production of nursery seedlings, after planting care is critical. In common with many other tree legumes, *C. calothyrsus* often displays slow early growth (Evans, 1984; Glover and Heuveldop, 1985; Jama *et al.*, 1989) which is likely to be linked to poor, or ineffective associations with soil microsymbiants. If good association is achieved,

directly sown plants have been reported to reach heights of 3.5 m in six months (Wiersum and Rika, 1992).

Direct sowing requires good land preparation, preferably ploughing, in order to reduce early competition from weeds. Seed should be sown at the start of the rainy season (immediately after the first heavy rains). Seed can be placed approximately 0.5-1 cm below the surface of the soil and covered with a fine soil layer. The main cause of mortality in direct sowing is seed predation, followed by competition from weeds. Weeding should be carried out regularly until the plants are established. At least three or four weedings may be required in the first season alone. Once seeds have germinated, any excess plants at a seed hole can be removed, leaving the healthiest individual.

In Java, encouraging results were obtained in trials where seed was scattered onto degraded grasslands, prepared either by burning, or ploughing, with 10% survival after 10 years (Hadipoernomo, 1979). Where land rehabilitation in remote sites is required, such methods may prove attractive, although resultant cover tends to be uneven. Where extensive stands of *C. calothyrsus* already occur, natural regeneration is often sufficient to maintain tree cover. Mangundikoro (1970) observed up to 500 seedlings per m^2 under breaks in the canopy of *C. calothyrsus* stands on Mount Banyak, Java. By thinning such stands, suitable conditions for plant growth can be obtained.

Nursery seedlings

Producing seedlings in the nursery provides higher survival than direct sowing as seed predation and weed competition upon planting out are reduced. The reliability of germination of fresh, or well stored seed of *C. calothyrsus* means that usually 75-90% of seed produces healthy plants. Seed can either be sown into containers, or directly into nursery beds to produce bare rooted seedlings.

Containers

For container grown plants, it is important that the selected containers are large enough to allow adequate root formation and free drainage. However, they should not be so large as to increase transport costs excessively. Black polythene bags are relatively cheap and their opacity reduces the growth of algae within the soil. Either 10 x 15 cm, or 10 x 20 cm bags are used widely and well suited to the purpose.

Soil mixture

The soil mixture should be sufficiently permeable to allow root penetration and water infiltration. Heavy clays should be avoided, since *C. calothyrsus* does not tolerate water-logging. The soil should have enough cohesion, however, to form a root ball that does not disintegrate on removal of the container. The soil should ideally have a pH between 6-8 (Hu *et al.*, 1983). An ideal medium is a 1:1 mixture of peat and vermiculite. Where this is unavailable, a good topsoil, or a 2:1 mixture of clayey soil and sand can be used, with organic matter added to increase water retention if necessary. If inoculum is not to be applied directly to the seed, soil should be collected from areas where *C. calothyrsus*, or some other leguminous tree species, is growing. This soil should be mixed in a ratio of 1:10 with the nursery mixture to increase the chance of appropriate *Rhizobium* inoculation.

Sowing

Seed should be sown at a depth equal to their length (i.e., approximately 1 cm). Two seed per bag is normally sufficient to ensure adequate germination (NRC, 1983). Extra seedlings can be removed to leave the most vigorous seedling in a bag. When required, the removed seedlings can be pricked out into another bag, if removed when the first adult leaves are just showing. In nursery beds for the production of bare root seedlings, seed is sown at a spacing of 15 x 15 cm, or 10 x 30 cm. By placing the seed at the correct depth and covering with a coarse washed sand or grit, better drainage can be achieved and damping-off prevented. The soil should be kept moist until germination, but not over-watered thereafter. Seeds of *C. calothyrsus* can be expected to germinate in 4-8 days at 25°C with the first leaves unfurling within 12 days (Diangana, 1984; Macqueen, 1993b).

Management

Young plants should be placed in an area under approximately 50% shade. As the plants develop, the shading can be reduced gradually to allow hardening-off prior to planting out. Regular watering should be undertaken, preferably early in the morning before daytime temperatures rise. The soil should be kept moist, but not water-logged, since damping-off fungi (e.g., *Pythium, Phytophthora* and *Rhizoctonia*) flourish under such conditions. Good soil drainage and nursery hygiene help to prevent such problems. *C. calothyrsus* grows rapidly in the nursery, and roots may perforate containers and root into soil beneath. To avoid this, roots should either be manually pruned, or plants should be placed on raised mesh supports to allow air pruning to take place. If neither is practicable, regular movement of plant position avoids extensive root growth into the soil beneath, or a gravel base in the

nursery my be more appropriate.

Planting out

Calliandra calothyrsus is fast-growing under appropriate nursery conditions if inoculated with *Rhizobium* and, preferably, VAM fungi. Seedlings are ready to plant out when they are 20-50 cm tall with a root collar diameter of 0.5-1.0 cm (Kartasubrata, 1996; NRC, 1983). If planting-out is delayed, taller plants should be cut back in the nursery to 10-30 cm immediately, and once again just before planting. Planting holes should ideally be twice the dimensions of the container (usually 30 x 30 x 30 cm). At the beginning of the major rainy season, plants should be root pruned if necessary and transported to the planting site as soon as possible. *C. calothyrsus* does not tolerate extremely arid environments with less than 600 mm annual rainfall, but in borderline areas, mulching and micro-catchments can improve survival (Khosla and Puri, 1993; Weber, 1977).

Once planted, seedlings should be protected from fire, pests, weed competition and browsing animals. In its' native range, *C. calothyrsus* is not heavily browsed by cattle when other more palatable fodder species are present (Arias and Macqueen, 1996), although this may differ between provenances and in different environments.

Bare root seedlings

The cost involved with the production of plants in containers can be reduced by raising seedlings directly in nursery beds as bare root seedlings. Not only can seedling containers be dispensed with, but also transport to the planting site is less expensive. Bare root seedlings need at least 2-5 months in the nursery until they have reached a height of 60-90 cm. Once the seedlings have reached the desired size, they can be drenched with water in the beds and then carefully uprooted and transported to the planting site. The critical factor in this procedure is to keep the plants' roots protected and moist until planting, either by smearing them with mud or by packing in moss. Bare rooted seedlings should be planted as quickly as possible after uprooting. The ideal planting time is at the start of the rainy season (after the first heavy rains), and planting holes should be sufficiently large to allow roots to hang freely without bending or spiralling. A hole size of 30 x 30 x 30 cm is usually adequate for such purposes.

Vegetative propagation

Due to limitations in the quantity of *C. calothyrsus* seed available for planting, vegetative approaches to

propagation have been assessed. *C. calothyrsus* cannot be propagated readily from leafless stakes planted directly in the ground, but other methods of low cost vegetative propagation have been investigated for the multiplication of *C. calothyrsus* plants (Dick *et al.*, 1996; Tchigio and Duguma, 1998).

In an experiment using low technology, non-mist, propagators in a tropicalised glasshouse in Scotland, Dick *et al.* (1996) investigated the rooting ability of leafy stem cuttings of six provenances of *C. calothyrsus*. The stem cuttings were taken from seedlings 25-30 cm tall. Soft apical tissue was discarded and 5-10 cm lengths were then cut from the remaining stem provided there was a healthy green leaf at the node. Multi-node cuttings were used if the leaves were spaced at less than 5 cm. Leaves were trimmed to four pinnae pairs and the cutting base was treated with commercial rooting powder (containing 0.8% indole butyric acid (IBA)) before placement in 0.01-12.7 mm quartz grit rooting medium in a non-mist propagator. The propagator bed temperature was maintained between 20-28°C (air temperature 21-32°C) with 70% shade except on overcast days. Humidity was maintained above 95% except when opening the propagator lid. From 247 cuttings, 55% formed adventitious roots after five weeks (with 97% of all rooting activity within four weeks). There was significant variation between provenances from 0% for Patulul, Guatemala to 76% for San Ramón, Nicaragua.

Tchigio and Duguma (1998) also undertook the vegetative propagation of *C. calothyrsus* in non-mist propagators of the same design as used by Dick *et al.* (1996). In two separate experiments, they tested the effect of variation in rooting medium, and the effect of different growth hormones on rooting ability. Juvenile shoots were harvested from *C. calothyrsus* grown in Cameroon, and 4 cm long single node cuttings were removed from the shoots and transplanted to the propagator. The propagator bed temperature was maintained between 25-27°C, and relative humidity maintained at 80%. The formation of roots was obtained after four weeks, but varied according to the rooting media and hormone treatment. The most successful result was achieved with a rooting medium of a 1:1 mixture of fine sand and rotted sawdust, both in terms of the development of roots and shoots. The addition of growth hormones had little overall effect on the percentage of rooted cuttings, but the addition of IBA did have a significant effect on the mean number of roots produced, their lengths and dry weights.

Plants grown in mist propagators are susceptible to rotting because of high humidity. Tomaneng (1991) rooted cuttings of *C. calothyrsus* with two nodes within 14 days in a mist propagator using IBA rooting powder, but had to control rotting using two applications of benelate spaced by a ten day interval. In the light of these, and the author's own results, Dick *et al.* (1996) concluded that non-mist propagators may be more effective than mist propagators for *C. calothyrsus*.

While vegetative propagation is a possibility for the mass multiplication of *C. calothyrsus*, careful consideration should be given to the genetic diversity of the resultant plants. Furthermore, for other tree species, e.g., *Gliricidia sepium*, rooted cuttings do not appear to perform as well as seedlings on poor soils, both in terms of survival, and resilience to coppicing (Evensen *et al.*, 1994; Maclean *et al.*, 1992). To date, propagation of *C. calothyrsus* has been undertaken principally from seed. However, where seed production yields continue to be poor, the use of vegetative propagation may be an important option.

Recommended mode of propagation for *C. calothyrsus*

♦ Where possible, propagate *C. calothyrsus* from seed.

♦ Where labour permits, nick the coat of individual seeds opposite the gap in the horse-shoe shaped marking on the seed coat. Alternatively, soak seed for 10 minutes in water at 70°C followed by 12-24 hours in cold water.

♦ Inoculate the seed with a cocktail of approved *Rhizobia* by applying 50 g of inoculum to each kg of seed using vegetable oil or a sugar solution as glue.

♦ Sow two seeds in a 10 x 20 cm container and remove the second seedling after its first adult leaves are showing.

♦ Place seedlings under 50% shade and gradually reduce shade prior to planting, lifting containers occasionally and pruning the roots.

♦ When seedlings are 20-50 cm tall with a root collar diameter of 0.5-1 cm, transfer to the planting site and plant out immediately, but only after the first heavy rains.

♦ Plant in holes 30 x 30 x 30 cm and protect from fire, browsing animals and pests.

♦ If the planting season has been missed, cut the plants back immediately to 10-30 cm tall and again, just before planting, in the subsequent planting season.

Plate 3

A -   Seedlings of *Calliandra calothyrsus* in the nursery.

B -   Extracting seed from mature, golden brown pods of *Calliandra calothyrsus*.

C -   A cluster of mature pods of *Calliandra calothyrsus*.

D -   Mature pod of *Calliandra calothyrsus* showing the dark brown seeds.

E -   A seed production orchard in Australia where the *Calliandra calothyrsus* trees are planted in widely spaced rows.

5   Use and management

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5.1   Introduction

Like many agroforestry trees, *C. calothyrsus* has a multiplicity of uses. The rapid spread of the species from its native range in Central America and Mexico to countries throughout the humid tropics is evidence of this versatility. High growth rates in appropriate climatic conditions are complemented by vigorous and repeated re-sprouting after cutting, allowing harvests of leafy or woody material over many years, and the easy manipulation of tree height and form to reduce or increase above and below ground interactions with associated crops.

While the predominant use of *C. calothyrsus* is increasingly the production of supplementary fodder, other uses are also found within different farming systems and include the provision of green manure, fuelwood, shade or support for perennial crops, land rehabilitation and erosion control, and honey or shellac production.

The species has particular relevance in the humid tropics and on acidic soils where other agroforestry trees perform poorly. It is primarily used outside its native range, in areas where high population density places pressure on limited land resources, and where agroforestry systems are being developed to maintain and sustain agricultural production. The purpose of this chapter is to describe the uses of *C. calothyrsus,* and the optimal regimes for their management.

5.2   Fodder

Calliandra calothyrsus has been recognised as a useful fodder tree in areas where the use of other multipurpose tree species is limited by environmental or disease constraints. For example, the widespread destruction of *Leucaena leucocephala* by the psyllid, *Heteropsylla cubana*, prompted a search not only for resistant species within the genus *Leucaena*, but also for other multipurpose trees adapted to tropical and sub-tropical regions. The tolerance of most provenances of *C. calothyrsus* to acid soils is an important attribute which has encouraged their use as

fodder trees in animal production systems in the humid tropics (Berhe and Mohamed-Saleem, 1996; Palmer *et al.,* 1989; Ty, 1996). Although there is clear evidence that *C. calothyrsus* may produce yields of fodder comparable with that of *L. leucocephala* (Palmer and Ibrahim, 1996), the nutritive value of its leaves appears to be highly variable and usually inferior to that of *L. leucocephala*. In addition, studies on the value of *C. calothyrsus* as a fodder tree have been, until recently, hampered by the availability of only a limited range of germplasm. The International Workshop on the Genus *Calliandra* held in 1996 in Indonesia brought together the latest information on the production, utilisation and nutritive value of *C. calothyrsus* from many different countries (Evans, 1996), and the following sections draw heavily on this workshop for their information and conclusions.

Chemical composition

The chemical composition of feeds is often the first evaluation that is made in relation to nutritive value. Table 5.1 shows the range of values for the protein, fibre (neutral detergent fibre [NDF] and acid detergent fibre [ADF]), lignin, condensed tannin and ash contents of oven-dried samples of *C. calothyrsus* which have been obtained by Ahn *et al*. (1997), Jackson *et al.* (1996), Merkel *et al.* (1996), Norton (1994a) and Shelton *et al*. (1996). For comparison, values for *L. leucocephala* are included. Also shown are values for *in vitro* dry matter digestibilities (IVDMD) which are crude measures of availability of these nutrients for digestion and absorption by ruminants. The wide variation in some of the measurements can be accounted for in part by differences between accessions or provenances, the effects of environment on leaf composition, and to differences in the analytical techniques used.

Table 5.1:   Range of values reported for the chemical composition (g/kg dry matter) and *in vitro* dry matter digestibilities (%) of oven dried *Calliandra calothyrsus* and *Leucaena leucocephala*.

Species	Crude protein (Nx6.25)	Neutral detergent fibre	Acid detergent fibre	Lignin	Condensed Tannin	Ash	*In vitro* digestibility
Calliandra calothyrsus	173-282	259-599	209-504	69-245	15-194	40-59	19.3-48.4
Leucaena leucocephala	203-269	309-467	226-271	68-138	37-55	46-93	60.1-68.8

With the exception of the nitrogen (N) and sulphur (S) content of proteins, there is little other information available on the chemical composition of *C. calothyrsus* leaves. It would seem unlikely, however, that *C. calothyrsus* leaves would differ greatly from other leguminous tree leaves in their elemental composition, and generally tree leaves provide a rich source of minerals to the diets of grazing animals in the tropics. The protein content of *C. calothyrsus* is high in comparison with tropical grasses and some legumes, and similar in range to that of *L. leucocephala.* In Burundi, Akyeampong and Dzowela (1996) found that mixed hedges of a grass, *Tripsacum laxum*, with either *C. calothyrsus* or *Leucaena diversifolia*, provided fodder of a higher nitrogen and protein content than pure *T. laxum* hedges in the diet of goats.

Differences between provenances

The variation in fodder characteristics between provenances of *C. calothyrsus* has been studied by Palmer and Ibrahim (1996) in northern Australia (2 sites, 19 provenances), by Berhe and Mohamed-Saleem (1996) in the highlands of Ethiopia (3 provenances), and by Larbi *et al*. (1998) in Cameroon (14 provenances). In the Australian study, IVDMD of dried leaf material varied from 24 to 47%, and changes in N digestibility followed similar trends. These authors noted that despite a three-fold change in condensed tannin (CT) content between provenances, there was no clear relationship between CT and N digestibility. In the Ethiopian experiment, IVDMD decreased as cell wall (NDF and ADF) and lignin content increased, and there also appeared to be only a weak relationship between proanthocyanidin (tannin) content and N digestibility. In the experiment conducted with provenances planted in Cameroon (Larbi *et al*., 1998), there were highly significant differences between provenances for DM degradation. Degradable DM varied from 280 to 637 g/kg dry matter, and was inversely proportional to the rate of degradation which varied from 0.023 to 0.01 %/hr.
The variation in chemical composition and DM characteristics observed between provenances may be associated with differences in leaf anatomy, with provenances having low leaflet/petiole ratios having high cell wall, lignin and tannin contents and consequently lower digestibilities and potential nutritive values. This hypothesis could be tested through the further evaluation of provenances of *C. calothyrsus* in terms of leaf morphology, chemical composition and digestibility in as many environments and management systems as possible. Further research may also establish the relationship between condensed tannins and N digestibility.

Effects of drying on chemical composition

When considering the effects of drying, whether on chemical composition or nutritive value, it is important to differentiate between wilting, or air drying at ambient temperature (25-30°C), and oven drying at higher temperatures (typically 50-60°C). While air drying reflects one way in which fodder may be used, particularly in cut-and-carry systems, oven dried leaves are never used by farmers as fodder. The only reason for investigating the extent to which oven drying affects chemical composition is that leaves are often prepared in this way for analysis. Many studies have compared oven dried leaves with freeze dried ones (which are generally assumed to be equivalent to fresh material).

In general, the oven drying of plant tissue initially increases the rate of respiration from soluble carbohydrates, and when compared with fresh or freeze dried samples, there is an apparent increase in the fibrous components of the plant dry matter. Drying also denatures and precipitates cellular proteins and promotes the binding of tannins to cell components. Oven drying *C. calothyrsus* increases the concentrations of NDF, ADF and lignin in the dry matter (Merkel *et al.,* 1996), but decreases apparent condensed tannin content (Ahn *et al*., 1989; 1997; Merkel *et al*., 1996; Palmer and Ibrahim, 1996; Shelton *et al*., 1996). It is relevant to note that drying increases not only lignin in the plant DM, but also the proportion of lignin bound in the plant cell wall (ADF), which may explain why cell wall

digestibility decreases on oven drying. There is also an analytical problem associated with the estimation of lignin in plants containing condensed tannins. The acid insoluble fraction isolated as lignin will contain bound tannins, and hence will be designated incorrectly as lignin. It is possible that the observed increases in lignin with drying are simply additional condensed tannin complexed to the cell wall during drying. Shelton *et al.* (1996) found that drying the leaves of *C. calothyrsus* decreases the total condensed tannin by 15% (77 to 65 g/kg) and increases the proportion bound to protein and cell wall fibre from 15 to 21%. The amount bound to protein was found to increase with drying, but there was no change in the amount bound to the fibre fraction.

It is clear that the condensed tannins present at high levels in *C. calothyrsus* are affected by oven drying, and that this can also affect estimates of fibre and lignin owing to changes in the binding of tannins to the cell wall. Even air drying at 30ºC significantly reduces the level of extractable tannin and increases the amount bound to protein, though without concomitant increases in apparent fibre content (Stewart *et al.*, 2000). It is therefore important to use freeze dried leaves of *C. calothyrsus* for analytical studies, especially those involving condensed tannins.

Tannins and secondary plant metabolites

Although there is evidence that some *Calliandra* species contain secondary metabolites such as glycosides, saponins and flavonoids (Norton, 1994c), condensed tannins appear to be the metabolites of major significance in *C. calothyrsus*. There is presently no evidence that hydrolysable tannins (HT) are active in *C. calothyrsus*, but this area requires further study.

Although the mode of action of tannins in protein precipitation is thought to be well understood, little is known about the chemical nature and biological activity of tannins from fodder trees such as *C. calothyrsus*. There are a variety of methods which have been used to estimate the concentration of condensed tannins in plants, but until recently, there has been no common method which readily permits the comparison of tannin activity in different plants. For example, the vanillin-HCl technique gives a value of up to twice that of the butanol-HCl method (Jackson *et al.*, 1996). A complication with using the butanol-HCl method to compare provenances of *C. calothyrsus* arises from the considerable variation in condensed tannin structure (ratio of catechin/epicatechin to gallocatechin /epigallocatechin) within the species (Stewart *et al.*, 2000). This results in provenance variation in colour yield per unit mass of tannin with this method.

The extraction techniques for tannins from plant tissue have also varied significantly, and it is now accepted that both bound (to protein and fibre) and soluble condensed

tannin should be estimated in any analysis of plant tannin composition. There is an urgent need to establish standard analytical techniques for condensed tannins that describe the concentrations of these biologically active molecules, and thereby permit a better understanding of the role of tannins in the nutrition of the animal.

The effects of tannins may be modified by the inclusion of polyethylene glycol (PEG) in the animal diet. PEG preferentially binds to tannins rather than the dietary constituents in leaf matter, thereby overcoming any detrimental effects on digestion and utilisation. Palmer *et al.* (1994b) found that PEG significantly improved the digestibility of dried *C. calothyrsus* leaves (40% diet) in sheep, but did not improve the digestibility of the fresh leaf. This finding suggests that the tannins in fresh leaf were not active, and were only activated by the process of drying. However, when PEG was supplied with either frozen (fresh) or dried *C. calothyrsus* leaves, provided as a supplement (20% DM) to sheep given barley straw, PEG increased N digestibility for both forms of supplement without affecting overall DM digestibility (Norton and Ahn, 1997).

Nutritive value

The nutritive value of a feed is an estimate of the net availability of nutrients to an animal, which in turn, determines the extent to which a feed might promote animal production (growth rate, milk production, wool growth, etc.). The components of nutritive value are voluntary feed consumption (VFC) and the digestibility (D) of the feed, the product of these components providing a measure of nutrients available for animal production. In ruminants, VFC is determined by both animal (physiological state, palatability, hunger/satiety signals) and plant (physical and chemical) factors, while D is largely determined by the composition of the feed. Palatability refers to the animal's preference to consume, and is often related to the presence of specific attributes of feeds such as smell, taste and physical structure (e.g., hairiness), and not necessarily related to gross chemical composition and digestibility.

Palatability, intake and digestibility

Freshly harvested leaves of *C. calothyrsus* have been found by the majority of researchers to be highly palatable, particularly where livestock have had access to this forage previously (Palmer and Schlink, 1992; Paterson *et al.*, 1996; Shelton *et al.*, 1996). There may be, however, intra-specific differences in palatability. Palmer and Ibrahim (1996) found that although all the 19 provenances of *C. calothyrsus*

tested in two trials in Australia were accepted by cattle, some provenances were preferred over others.

Palatability, digestibility and intake of *C. calothyrsus,* when used as a sole feed, may also be depressed when the leaves are wilted or dried. Palmer and Schlink (1992) found that voluntary consumption decreased by 37% (from 59 to 37 gDM/kg$^{0.75}$/day) and *in sacco* digestibility almost halved when leaves were wilted. A detailed study of 19 provenances of *C. calothyrsus* subsequently found *in sacco* DM digestibility values in the range 59-76% for fresh leaves, reduced to 24-47% on oven drying. N digestibility was also reduced, from 25-80% when fresh, to 5-30% when oven dried (Palmer and Ibrahim, 1996).

When offered as a supplement (the usual way in which *C. calothyrsus* is used as a fodder), however, this reduction in quality on drying is no longer apparent. Palmer and Ibrahim (1996), feeding *C. calothyrsus* as a supplement (~28%) to buffelgrass, found that wilting had no significant effect on either whole tract digestibility or feed intake in sheep. Norton and Ahn (1997) found that drying *C. calothyrsus* actually improved its value as a supplement for sheep given barley straw, and more low quality straw was consumed by sheep given dried compared with fresh (frozen) *C. calothyrsus* leaves.

Animal production

There is a range of strategies that have been used to feed fodder tree leaves to ruminants, varying from the cut-and-carry systems of subsistence farmers to the extensive grazing of trees as part of a range management system. Although fodder tree leaves are used to feed cattle, sheep and goats in the tropics, it is not known whether these species differ in their capacity to utilise *C. calothyrsus*. Evidence available suggests that sheep and goats utilise *C. calothyrsus* as a dried supplement with equal efficacy (Perez-Maldonado and Norton, 1996). It is likely that cattle will respond similarly to sheep and goats when offered *C. calothyrsus* as a source of feed.

Use as a supplement to low quality diets

Tree legume leaves are commonly fed to improve the utilisation of low quality grasses and crop residues in tropical areas. For example, goats in Zambia maintained on poor quality pasture hay lost weight (-20 g/day), but gained weight (24 g/day) when provided with supplements of *C. calothyrsus* (140 g DM/day (Waterfall, 1993). The response of animals to supplements will depend on the composition of both the basal hay and fodder tree leaves, and on the level of supplementation (Norton, 1994b). Palmer and Ibrahim (1996) found that increasing levels (0 to 35% DM) of fresh *C. calothyrsus*

leaves in a diet of low quality hay increased the live-weight gain of sheep from -27 to 52 g/day. Table 5.2 shows mean values for the effects of increasing levels of dried *C. calothyrsus* leaves on the intakes of barley straw and weight gains of goats held in pens (Waterfall, 1993). This table demonstrates a number of important points about supplementary feeding of fodder legumes to ruminants.

It is well known that barley straw is deficient in N and the provision of urea in molasses is a low cost alternative for improving nutritive value. It is this treatment which is compared with varying levels of *C. calothyrsus* supplementation, and it was found that urea/molasses was more effective than any supplement for improving barley straw utilisation. At the lowest level of supplementation, straw intake was maintained and *C. calothyrsus* was consumed as additional feed, but at higher levels there was a direct substitution of straw by *C. calothyrsus*. This study also showed that while 35% of *C. calothyrsus* leaves in the diet promoted an overall increased intake and performance, levels higher than this decreased intake, and when offered as the sole diet, was less effective that at the lowest level of supplementation. It was concluded from this study that feeding *C. calothyrsus* did improve the nutritive value of low quality diets for ruminants, but was similar to feeding urea/molasses of equivalent N and energy content.

There is a widespread assumption that wilting or drying *C. calothyrsus* has a severe detrimental effect on its quality, presumably deriving from the studies showing a marked reduction in *in sacco* digestibility on drying (Mahyuddin *et al*., 1988; Palmer and Schlink, 1992). The few animal production experiments designed to compare fresh and wilted leaves have not, however, tended to support this assumption. Palmer and Ibrahim (1996) found no significant difference between fresh and wilted *C. calothyrsus* fed as a 28% supplement to buffelgrass on live weight gain and wool production in sheep. Unpublished results from trials in Kenya have also shown no effect of wilting on either live weight gain in sheep, or milk production in dairy goats when *C. calothyrsus* was fed as a supplement to Napier grass (J.N.N. Kangara and J.L. Stewart, unpublished results).

Table 5.2:   Mean values for feed intakes, digestibilities and live-weight changes of goats given varying levels of dried *Calliandra calothyrsus* leaves as supplements to low quality barley straw (Waterfall, 1993).

Level of supplementation (gDM/kg live-weight)	*C. calothyrsus* intake as % dietary DM	Voluntary feed consumption (g/kg$^{0.75}$/day)		Dry matter digestibility (%)	Nitrogen balance (g/day)
		Straw	Total		
0*	0	35.1	41.5	53.1	1.4
0.72	35	31.5	48.6	53.8	-0.7
1.27	58	21.9	52.4	57.1	-0.1
Ad libitum	100	-	45.7	47.4	-2.4

* 30 g urea plus 70 g molasses supplied with basal diet.

Use as a replacement for concentrates

The leaves of *C. calothyrsus* are high in protein and mineral content and can act as a substitute for some of the more expensive components of concentrate supplements used in dairy and feedlot rations of intensively reared livestock. Paterson *et al.* (1996) found that for dairy cows under East African conditions, 3 kg fresh *C. calothyrsus* leaves provided a similar response in milk yield and butterfat per cent to 1 kg of commercial concentrate. This response was obtained by adding either 3 kg of fresh *C. calothyrsus*, or 1 kg concentrate, to the 2 kg of concentrate already given with the diet. It is likely that this feeding regime does not provide additional energy and rumen degradable protein, but may provide a source of tannin-protected protein that has an effect on milk yield and quality by increasing the amount of intestinally absorbed protein in these cows. However, when concentrate or *C. calothyrsus* was used in the same relative proportions as a supplement to Napier grass in the diets of sheep and goats (without additional concentrate), the concentrate gave significantly better animal performance that the *C. calothyrsus* (J.N.N. Kangara and J.L. Stewart, unpublished results).

In Sri Lanka, Perera and Perera (1996) studied the effects of substituting rice straw in the diet of goats with 10-50% *C. calothyrsus* leaves in coconut oil meal. These researchers found that up to 30% of this concentrate could be replaced with *C. calothyrsus* without affecting feed intake. Both *Leucaena leucocephala* and *Gliricidia sepium* have been successfully incorporated into tropical grass silages (Tjandraatmadja *et al.,* 1993), and it is possible that *C. calothyrsus* leaves may also be a valuable source of protein and minerals for silages.

Use in non-ruminant diets

Some fodder tree legumes have been used to replace protein, and provide a source of minerals and vitamins in pig, poultry and rabbit diets. The major limitations to their use, however, are the high fibre content of leaf material, which restricts the level of inclusion, the low energy content, and the presence of tannins. Paterson *et al.* (1996) fed *C. calothyrsus* leaf meal to chickens over a short period of time. Inclusion rates varied from 0 (commercial ration) to 15%, and they found that while there was no significant effect on feed intake, or average egg weight, inclusion rates greater than 5% decreased the number of eggs produced per chicken. Yolk colour was markedly improved at the 5% level, and it was concluded that 5% *C. calothyrsus* leaves was the maximum level of inclusion consistent with the maintenance of production.

Use in grazing systems

There have been few trials that have evaluated *C. calothyrsus* as a forage directly grazed by ruminants, although such studies have been made for other tree legumes (e.g., *Leucaena*, *Sesbania*). Palmer and Ibrahim (1996) have reported the results of a trial in which cattle, grazed on *C. calothyrsus*/Signal grass (*Brachiaria decumbens*) pastures (5 steers/ha), gained 1 kg/day over 40 days, indicating some potential for the use of the species in tropical grazing systems. Although *C. calothyrsus* has been found to respond well to frequent cutting (Gutteridge, 1990), there is accumulating evidence that plants can be damaged by direct grazing. Kochapakdee (1990) found that when goats grazed mature stands of *C. calothyrsus* pruned to 1 metre, they damaged the

bark of some trees, which resulted in a 36% mortality of trees following grazing. In the same trial, *Albizia chinensis* and *Leucaena leucocephala* suffered little damage or mortality, but *Sesbania sesban* and *Gliricidia sepium* were severely de-barked and had mortalities of 86 and 44 % respectively. This was not simply an effect of goats, as sheep were observed to cause similar damage in later studies with the same trees. In Bali, Indonesia, cattle grazing *C. calothyrsus* stands (3 steers/ha) also removed layers of bark and cambium, and within two years of establishment all plants had died (Rika *et al.,* 1995). This poor persistence under grazing is a serious limitation to the use of the species in extensive grazing systems, and is an attribute that should be evaluated in the provenances currently being tested for use in tropical areas.

Information on the fodder value of *Calliandra* is largely limited to *C. calothyrsus*, although the recent collection and characterisation of other *Calliandra* species by OFI provides an opportunity to review, test and select species with better forage characteristics.

Current evidence suggests that fresh *C. calothyrsus* is highly palatable to ruminants, but intakes and digestibility (nutritive value) may be limited by high condensed tannin contents. Drying *C. calothyrsus* appears to depress intake and digestibility when fed as a sole source of feed, but drying may improve nutritive value where the species is used as a supplement to a low quality basal feed. The presence of high concentrations of condensed tannins possibly confers a beneficial effect by protecting proteins from ruminal degradation, and a detrimental effect by binding to plant cell walls and inhibiting their digestion by fermentative organisms in the rumen of grazing animals.

Management for fodder

Studies on fodder production have been conducted under varying environmental conditions using a range of management practices. The most usual planting arrangements for *C. calothyrsus* as a fodder source have been in hedgerows, or some other form of line planting. On sloping land, these hedgerows have invariably served the additional function of soil erosion control. Occasionally trees are planted as blocks on fallow land and fodder is cut and carried to penned livestock, or trees are grazed directly (e.g., in Australia; Palmer and Ibrahim, 1996). The implications of these different systems are discussed below.

Many farmers in the tropics use *C. calothyrsus* in cut-and-carry systems where leaf material is cut and offered to penned livestock, often in mixtures with other forages (Mullen *et al*., 1997). In these systems, hedgerow plantings at a spacing of 50 cm between plants within rows was found to be optimal in terms of fodder production, when the initial cost of establishment is taken

into consideration (Akyeampong, 1996; Baggio and Heuveldop, 1984). In density trials using fodder blocks, both leaf and wood yield were positively correlated with plant density in the wet, but less so in the dry season, with maximum production using a 12 week cutting frequency (Ella *et al.*, 1989). Paterson *et al.* (1996) and Shelton *et al.* (1996) have also recommended cutting frequencies of 8-12 weeks.

The timing of the first cut upon subsequent fodder production is also important. Ella *et al.* (1991) have demonstrated a positive correlation between tree age at first cutting and subsequent leaf biomass yield, and suggested that cutting of *C. calothyrsus* can start after a relatively short 12 month establishment phase. Akyeampong and Muzinga (1994) suggested that the timing of pruning is important for the quantity of leaf biomass that can be harvested in the driest months of the year (when the need for fodder is correspondingly greatest). In Burundi, the optimal cutting time was six months before the middle of the dry season.

Cutting height is another factor which affects yield, and research in Africa has demonstrated that for *C. calothyrsus*, in common with other agroforestry trees, production increases with increasing cutting height from 0.1 to 1.3 m (ICRAF, 1992). Paterson *et al.* (1996) suggested the most productive cutting height to be 0.6-1.0 m. The height of cutting also affects the browsing habits of animals in direct grazing systems. Kochapakdee (1990) found that *C. calothyrsus* suffered relatively high mortality when grazed by goats. Where the fodder plants had been cut to a height of 1.0 m before grazing, however, goats and sheep caused little damage (Callow, 1993). This relates to an experiment in Bali where cattle were allowed to graze well established *C. calothyrsus* (2.3 m tall) and stripped layers of bark and cambium causing 100% plant mortality within two years. B. Palmer (pers. comm.[1]) has suggested that in situations where the cutting height is low, cattle are unable to strip the bark and plant mortality is reduced. Grazing habits may therefore reduce the optimal cutting height for fodder production.

Where *C. calothyrsus* is grown in combination with grasses for the production of animal feed, tree-grass interactions potentially play an important role in overall yields. In an experiment at Gowa, south Sulawesi, Catchpoole and Blair (1990) demonstrated that the growth of grass, *Panicum maximum*, was highest in pure plots and was reduced by the presence of agroforestry trees. The reduction under *C. calothyrsus* was less than that under *Leucaena*

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leucocephala or *Gliricidia sepium*, which reflected their higher biomass production. The authors concluded that there was little N transfer from the legume trees to the grass, but that the total N yield in the different systems rose from 103 kg/ha in the pure grass control to 901 kg/ha in the tree-grass mixtures.

Management of *C. calothyrsus* for fodder production

♦ After accustomization period, feed 30-40% *Calliandra* in a mixture of grass or other browse species.

♦ Plant at high densities (25-50 cm within hedges, or 50 x 50 cm to 1 x 1 m in fodder banks).

♦ Allow plants 1 year to establish before first cut.

♦ Cut at 8-12 week intervals.

♦ In direct grazing systems, cut trees back to 0.6-1 m above the soil surface.

♦ Feed fresh (either by direct grazing, or by minimising time between cutting and feeding), or dry as a supplement in the diet.

5.3   Green manure

As early as 1941, the Dutch colonial forest service in Indonesia highlighted the potential of *C. calothyrsus* for use as a green fallow, or in mixtures with crops, for the supply of green manure (Verhoef, 1941). The species has since been evaluated when inter-planted with crops and in soil erosion control hedges, particularly on the sloping acidic soils of humid, sub-montane regions of Indonesia (Rourke and Suardika, 1990). Elsewhere in the tropics, the use of *C. calothyrsus* for the provision of green manure has also received favourable reports. For example, in Cameroon, a 52% increase in grain yields between *C. calothyrsus* hedgerows in on-farm trials prompted 52 farmers to join a programme of alley cropping evaluation (Tonye *et al.*, 1995). Contrasting reports, however, have shown that the species can be associated with poor crop yields (Heineman, 1996; Heineman *et al.*, 1997; Macklin, 1990).

Tree-crop mixtures have the dual benefits of N-fixation by the tree legume and the supply of leaf material for mulching the associated crop. Because of the contrasting reports surrounding the performance of *C. calothyrsus* in mixtures with crops, careful evaluation of the species' potential for this use within such systems is needed. The following factors contribute to the suitability of *C. calothyrsus* for planting with crops: (1) above ground competition (mainly for light); (2) below ground root competition (mainly for water and nutrients); (3) changes in soil physical properties; and (4) nutrient concentrations in leaf tissue and their decomposition and recycling.

Above ground competition

The high growth rates and productivity of *C. calothyrsus* in many parts of the tropics are not necessarily beneficial for tree-crop interactions. Brook (1992) reported that the growth rate of *C. calothyrsus* hedges in an alley cropping experiment in Papua New Guinea depressed the yield of sweet potato through shading prior to pruning. In addition, its brittle branches tended to break and fall over the sweet potato plants, causing some damage to the crop. Evensen *et al.* (1995) also noted the problem of crop shading by *C. calothyrsus* hedges in west Sumatra, where the species gave high leaf yields of up to 3.18 t/ha/year. Hariah *et al.* (1992) observed that on an ultisol in Sumatra, whilst *C. calothyrsus* hedges spaced at 4 m produced more biomass and supplied more nitrogen than a number of other species, they needed regular intensive pruning to avoid shading of the inter-crop. The authors suggested greater inter-row spacing to reduce this problem.

Below ground root competition

Dierolf *et al.* (1989) described the root distribution from *C. calothyrsus* hedges in an alley cropping system, including the roots of the cowpea crop. They found that 78% of the total fine root weight was found in the top 0-15 cm of soil with a further 9% in the 15-30 cm layer immediately beneath. Roots were still obtained at 150 cm depths (below the depth normally associated with food crops) suggesting that the tree may be able to recycle nutrients from deeper soil levels. The more vigorous tree hedges had markedly more roots at deeper levels (below 1 m) than slower growing hedges. Such results confirm the early root excavations of Verhoef (1939), whose drawing of the root architecture of *C. calothyrsus* is shown in Figure 5.1.
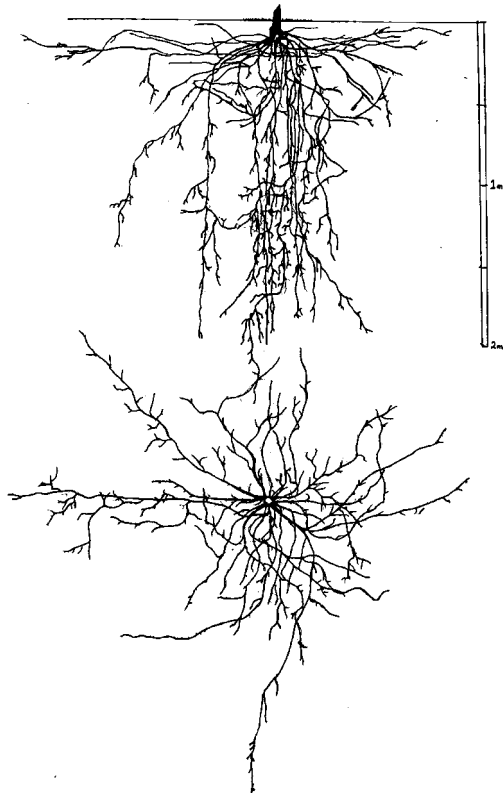
Figure 5.1: Root architecture of *C. calothyrsus* (from Verhoef, 1939).

Hariah *et al.* (1992) found that the roots of *C. calothyrsus* were largely restricted to the top 10 cm of the soil with few major roots below 1 m, and that the species had higher numbers of branch roots, but of a thinner diameter than comparable agroforestry trees. A field experiment on a shallow, sandy plinthic/ferric acrisol in Togo under dry climatic conditions demonstrated that the yield of maize grown next to a hedge of *C. calothyrsus* was depressed in comparison with hedges of *Cassia siamea* or *Gliricidia sepium* (Schroth and Lehmann, 1995). These authors also found that root barriers between *C. calothyrsus* and the crop greatly increased maize yields, thus highlighting the competitiveness of the species' root system. Likewise, Sitompul *et al.* (1992) demonstrated that root pruning of three-year old *C. calothyrsus* was beneficial in alley cropping experiments.

Rosecrance *et al.* (1992a) in their study of nine leguminous tree species inter-cropped with maize observed that competition with maize was a function of the tree growth rate, and that this was independent of the species. Heineman (1996), in his investigation of the effect of *C. calothyrsus* hedges on maize yields on a gleyic cambisol in western Kenya, noted that the high productivity of the species was associated with strong competition within hedgerows for both water and, possibly, nutrients. This competition significantly reduced the positive nutritional effect of the mulch on crop yields. Inevitably, tree-crop interactions depend on

both the tree species and crop species. For example, when *C. calothyrsus* was planted in association with banana in the highlands of Burundi, there was no significant reduction in banana yields (Akyeampong *et al.*, 1995). When planted under coconut plantations in Sri Lanka, the species produced 745 kg/ha of leaf biomass, and the green mulch raised the yield of nuts by up to 24% through a combination of improved soil physical, biological and chemical properties (Liyanage and Abeysoma, 1996).

Changes in soil physical properties

The changes in soil porosity associated with the roots of agroforestry trees are well documented. In Réunion, Cattet (1996) and Tassin *et al.* (1995) reported effects up to 50 cm distant from *C. calothyrsus* hedges with increased pore diameter and hydraulic conductivity at saturation, particularly uphill from the hedge. Rosecrance *et al.* (1992b) also recorded that four years of mulch application from *C. calothyrsus* hedges to crop rows measurably improved soil water holding capacity and bulk density. Conversely, small percentages of precipitation may be lost by the soil when rain is intercepted by tree species and evaporates. Pidjiharta and Pramono (1988) recorded percentages of rainfall reaching the surface soil of 56% under *C. calothyrsus* hedges as opposed to 64% for bare land after run-off and evapotranspiration. Vasquez (1987) recorded lower moisture content under *C. calothyrsus* pasture than under pure *Panicum maximum* pasture (42.7% and 51.4% respectively).

Hedges planted across sloping land serve the additional function of erosion control. In an experiment in Rwanda, Konig (1992) found that soil losses on bare fallow land (with a slope of 28%) totalled 55.7 t/ha/yr, whereas on agroforestry plots with *C. calothyrsus* hedges and the fodder grass *Setaria splendida*, losses amounted to less than 12.5 t/ha/yr. Such differences will inevitably have long term implications for soil fertility which are often not calculated in inter-cropping experiments.

Nutrient composition, release and recycling

Leaf and twig nutrient composition and the way in which these nutrients are broken down and released, have important implications for the usefulness of agroforestry species. Nyamai (1992) demonstrated that *C. calothyrsus* was second only to *L. leucocephala* in the rapidity with which 50% of the total oxidisable carbon was converted to CO_2. In an experiment by Handayanto *et al.* (1992), however, the decomposition rate for *C. calothyrsus* was slow in comparison with *Erythrina spp.* (taking

approximately 15 weeks before 50% of the total oxidisable carbon was converted to CO_2).

Nitrogen mineralization can also give an indication of the species potential in inter-cropping systems. Soluble polyphenols in the leaves are believed to prevent nitrogen mineralization during decomposition, and high levels have been found in the leaves of *C. calothyrsus*[2] (Constantinides and Fownes, 1994). Lehmann *et al.*, (1995) quote figures for polyphenol percentages of 13% in leaf material, 1.4% in twig material and 18.7-20.3% in root material. Lehmann and Schroth (1993) found that nitrogen release in the first weeks of an alley cropping experiment in Togo was inversely related to polyphenol:N ratios. In their experiment, *C. calothyrsus* had a higher concentration of polyphenols than either *Cassia siamea,* or *Gliricidia sepium,* both in shoot and root material. Using glasshouse experiments with prunings of *C. calothyrsus,* Handayanto *et al.* (1995) also found that polyphenol:N ratios most accurately predicted nitrogen mineralization. They also found that it was possible to reduce polyphenol concentrations in the leaves by increasing the supply of nitrogen to the *C. calothyrsus* plants. Ensuring that plants actively fix nitrogen may therefore be an important management issue. Other results which are supportive of low nitrogen mineralization in *C. calothyrsus* are those using pot and field trials based on the application of *C. calothyrsus* mulch, which gave yields of maize below those of the zero-fertiliser control (e.g., Gutteridge, 1990; 1992; Mathews *et al.,* 1992).

Rosecrance *et al.* (1992a) published results suggesting a linear relationship between the nitrogen yield from hedges applied as mulch and the resultant maize yield, independent of species and despite differences in leaf and root polyphenol concentrations. In their experiment, *C. calothyrsus* performed relatively poorly (4.9 t/ha/yr as opposed to 12 t/ha/yr for the most productive species). Heineman (1996), however, has suggested that each species, and each provenance within a species, has a unique relationship between nitrogen yield applied as mulch and subsequent grain yields. He noted that the high levels of polyphenols in *C. calothyrsus* leaf matter (which was applied to maize seedlings in pot experiments) reduced the positive effects of the application of the mulch. In contrast, Kettler (1997) found that the yield of beans treated with a mulch of *C. calothyrsus* was significantly higher than mulch obtained from the leaves of *Gliricidia sepium* or *Inga edulis.* This variability in nutrient release from *C. calothyrsus* leaf matter to crops may weigh against the use of the species in inter-cropping systems in certain circumstances.

Heineman *et al.* (1997) recorded that the status of soil N, P, K, Ca, Mg and S in alleys between hedges of *C. calothyrsus,* from which mulch was harvested and applied, was much less improved than in alleys between hedges of *Leucaena leucocephala, Gliricidia sepium* or *Sesbania sesban.* Similar results have been found by Rosecrance *et al.* (1992b) with no improvement in soil C, N, P, K, Ca or Mg in alleys between *C. calothyrsus*, and no increase in taro yields above the control for a 5 m inter-row spacing. Gichuru and Kang (1989) similarly found no change in soil chemical properties in a maize/cowpea alley cropping experiment in south-western Nigeria, but increased yields of maize (although not cowpeas) were observed. Heineman *et al.* (1997) also found *C. calothyrsus* mulch lowered soil pH and raised levels of soil aluminium. This is in direct contrast to a study carried out in Burundi by Wong *et al.* (1995). These authors found that *C. calothyrsus* prunings, harvested from pure stands and applied to test plots of maize and beans, decreased the concentration of aluminium in the soil relative to control plots. A corresponding increase in grain yield was also observed that was attributed to reduced aluminium toxicity in the test plots. Similarly, Noble and Palmer (1998) found *C. calothyrsus* ash (dried and ground leaf material) had the potential to cause least soil acidification in comparison to ash from *Leucaena* spp. or *Stylosanthes* spp.

Organic matter from *C. calothyrsus* appears to possess a complex decomposition pattern that may vary according to the production system the species is being used in. The species' competitive root system means that *C. calothyrsus* may be less effective at increasing the crop yield between hedges per unit mulch than other commonly used agroforestry species. The extra time and effort required to manage such a productive species, without any clear advantage in terms of crop yield, may suggest that *C. calothyrsus* is not suited for the sole supply of mulch, unless it is planted at a very wide spacing. Organic matter from the species may confer greater benefits when applied to pure crop plots, through an associated increase in soil carbon and reduced acid and aluminium toxicity.

Management for green manure

If *C. calothyrsus* is to be used to provide green manure, trees should be managed in such a way as to maximise benefits for the companion crop. Because of the vigorous above and below ground growth of *C. calothyrsus* under appropriate climatic conditions, the spacing of the tree hedges should be wider than for comparable agroforestry trees, e.g., 0.5 x 6 m may be appropriate.

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[2] Polyphenols cannot be equated directly with tannins. Although tannins contain phenolic groups, the term is restricted to those phenols which bind proteins (Mueller-Harvey, 1989).

Among the various management decisions that have to be made, the most important is related to the timing, frequency and height of tree pruning. Pruning not only supplies important nutrients to the associated crop through the application of mulch, but also reduces competition for light and simultaneously, the degree of below ground root competition (Hariah *et al.*, 1992). The times when pruning is likely to be most beneficial to the associated crop are during crop establishment and periods of high nutrient demand. Hence, hedges should be allowed to establish without pruning during the first year. Subsequently, the first pruning can be made just prior to crop planting, thus reducing disturbance to the newly planted crops (Jabbar *et al.*, 1992). Lehmann *et al.* (1995) have recorded that the peak period for nutrient demand in maize is four to six weeks after establishment, and that in order to synchronise nutrient release with crop uptake, mulch should be applied one or two weeks after establishing the crop, or at maize planting (Mafongoya *et al.*, 1996). In order to maximise the production of mulch while reducing above and below ground competition, a pruning frequency of 8-12 weeks is recommended (although this can be tailored to suit the individual tree growth rate at the planting site and labour constraints). Cutting height should ideally be at 0.6-1 m in order to reduce shade over the main crop and maintain productivity.

Management of *C. calothyrsus* for green manure

♦ Plant trees at a spacing of 0.5 x 6 m.

♦ Ensure nodulation by inoculating with appropriate rhizobial strains.

♦ Allow trees to establish for 1 year prior to first pruning.

♦ Prune trees 1 week prior to planting the crop.

♦ Disperse prunings as mulch over the surface of the cropping area.

♦ Prune regularly (at least every 8-12 weeks) to reduce competition for light, water and nutrients.

♦ Use a cutting height of 0.6-1 m to reduce shading of crops and promote productivity.

Prunings can be laid on the surface of the crop land as green mulch. In coconut plantations, prunings have been laid on the surface of the soil in a 2 m radius around the

base of each palm (Liyanage and Abeysoma, 1996). While mulch can also be incorporated into the soil, Mulongoy *et al.* (1993) report no discernible advantage in doing so and the extra labour costs are such that the practice is not recommended. Moreover, where termites are abundant, the incorporation of woody stems into the soil encourages their activity and promotes rapid breakdown of organic matter to the detriment of longer term crop growth (Lehmann *et al.*, 1995). If farmers wish only to apply the leaf material as mulch and use the woody material as a source of fuelwood, labour can be saved by placing all of the material on the crop land and collecting up the stems once the leaves have fallen off.

5.4   Fuelwood

Up to 90% of the domestic energy consumption in rural communities of tropical countries may be provided by fuelwood (Eckholm *et al.*, 1984). Nevertheless, tree planting aimed solely at fuelwood production is comparatively rare, because of the low value of the product and the ability to acquire fuelwood in most cases from adjacent forests, or as a by-product from trees planted for other reasons (FAO, 1986). Where *C. calothyrsus* is planted for fodder, soil amelioration, shade, etc., partial or complete coppicing of the species for fuelwood can be undertaken. Under such conditions, fuelwood is often an important subsidiary commodity within the farming system. Occasionally, specific fuelwood programmes have been developed, such as the Kenya Woodfuel Development Programme (KWDP), in which *C. calothyrsus* played a role (Chavangi *et al.*, 1985).

Attributes

The wood of *C. calothyrsus* is of intermediate density, having a specific gravity between 0.5 and 0.8. It burns well, giving off approximately 4600-4720 kJ/kg (Chantrasiri, 1988; NRC, 1983). It converts to charcoal with a yield of approximately 35% and the charcoal has a fuel value of 7200-7580 kJ/kg (Chantrasiri, 1988; NRC, 1983; Syachri, 1982). Estimates suggest that 1 ha of *C. calothyrsus* can yield 14 tons of charcoal (FPRI, 1977). Charcoal briquetting experiments have been conducted by Hartoyo *et al.* (1978) with good results. The wood is also suitable for use as a smoking fuel in the production of smoked sheet rubber, and demand for this use is increasing as old rubber tree timber is increasingly being sought by furniture manufacturers (Palmer *et al.,* 1994a). In a study to investigate the variation in wood density amongst provenances of *C.*

calothyrsus, I. Gourlay (unpublished data[3]) measured nine-month old wood samples from 15 provenances supplied by the IRA/ICRAF Project in Cameroon. He found significant variation (p<0.001) between the provenances with densities ranging from 0.80 g/cm^3 (Santa María, Honduras) to 0.66 g/cm^3 (Madiun, Indonesia).

Fuelwood production and management

It is rarely the case that *C. calothyrsus* is planted exclusively for fuelwood production. Consequently, it is slightly misleading to quote production figures for fuelwood based on trial results in which pure stands of the species were planted and harvested with only that aim. Nevertheless, such figures do give an indication of the potential for wood production in comparison with other well-known agroforestry species. Blair *et al.* (1988) quote fuelwood production figures over three years, the average of which was 2.45 kg/tree/yr, equivalent to 24.5 t/ha/yr at a 1 x 1 m spacing on an acid ultisol in south Sumatra. Kushalappa (1989) quoted figures for green-weight wood production of 2-10 kg/tree/yr in Karnataka over different seasons. These figures compare well with average yields of 8.1 kg/tree/yr calculated for trees grown in west Java (Widiarti and Alrasjid, 1987). The results of the International *Calliandra* Network co-ordinated by the Oxford Forestry Institute (Pottinger, 1996b) have indicated large provenance differences for wood biomass production at different sites (see Chapter 6). In Bandung, Indonesia, for example, a fuelwood trial in which trees were planted at 2 x 2 m spacing, gave biomass figures which ranged from 69% of the control (planted from seed of local origin) to 154% of the control (Hernawan *et al.*, 1996). A fuelwood trial in Cameroon using a 1 x 1 m spacing gave figures of almost 39 t/ha/yr for the most productive provenance (Duguma and Mollet, 1997). This provenance was 1.8 times more productive than the least productive provenance. There were also differences in the ratio of wood-to-leaf biomass (from 4.1 to 6.8) suggesting that different provenances may be suited either to fodder or to fuelwood production.

Calliandra calothyrsus coppices well and this suggests that woodlots of the species can be managed over a period of 10-15 years with harvests every 1 to 4 years, depending on the size of main stem required. In Indonesia, villagers often set aside abandoned agricultural land, or areas covered in *Imperata cylindrica* as fuelwood fallows. The establishment of *C. calothyrsus* rapidly shades out weeds and improves the soil physical and chemical properties. After the fuelwood harvest, land can be returned to agricultural use, or coppice re-growth can be managed to provide further harvests of fuelwood. In areas of central Java, the government forestry service

has planted areas of the species in buffer zones around timber plantations, and such areas are regularly coppiced for fuelwood.

As in the production of leaf material, the first cut of wood should only occur after a period of one year. The cutting height for fuelwood is lower than in the management of fodder hedges and is normally at 0.2-0.3 m. This lower cutting height not only captures a greater percentage of woody biomass, but also ensures that future wood production is concentrated on only a few major re-sprouts. Subsequent management will vary depending on the desired end product. If larger diameter wood is required in future harvests, it may be wise to thin the stand to just one main stem after one year, before completing the main harvest after a further 1-3 years.

Management of *C. calothyrsus* for fuelwood production

♦ Plant trees at a spacing of 1 m x 1 m to 2 m x 2 m.

♦ When desired stem size is reached, cut main stems 0.2-0.3 m above the ground.

♦ Allow leaves to drop off (or remove them) prior to extracting timber from the planting site.

5.5   Shade and support for perennial crops

Shade

The use of *C. calothyrsus* as shade for coffee is the only widespread use of the species in its native range in Mexico and Central America (Arias and Macqueen, 1996). The species' rapid growth and coppicing ability provides effective shade for young coffee plants, although *C. calothyrsus* is usually replaced by other shade species of larger stature in the genera *Inga, Erythrina* and *Gliricidia* after 2-3 years (CATIE, 1991). In Sri Lanka and Indonesia, the species has been used as shade for tea. Its nocturnal leaf folding reduces night-time humidity by allowing evaporation and this attribute reduces fungal diseases in comparison with other shade trees. In Indonesia, the species has also been used as a nurse tree for the early growth of *Agathis lorathifolia* in exposed plantations (Karjono and Riyanti, 1979). *C. calothyrsus* was planted in alternate rows between

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the timber species and served to suppress *Imperata cylindrica* and give shade and protection to the commercial crop.

Calliandra calothyrsus can be planted as shade for coffee at a spacing of between 3 x 4 m and 5 x 5 m (CATIE, 1986; Martinez, 1987). Within such a system it is possible to prune at intervals of six months and incorporate leaf material into the soil or, alternatively, to prune less frequently and obtain fuelwood from branches of greater dimensions (Martinez, 1987). Trees are pollarded at a height of 2-3 m so that re-sprouts are able to provide shade quickly after pruning. The process of cutting risks damage to the crop underneath, so that care should be exercised during pruning.

Other associations with perennial crops

In an experiment in Maharashtra, India, Shinde *et al.* (1993) examined the suitability of ten agroforestry tree species for use as host plants for sandalwood (*Santalum album*). Sandalwood is hemiparasitic and attaches to the roots of a host plant via root outgrowths (haustoria). Sandalwood will obtain a portion of the available soil nutrients via the host plant, and a portion through its own roots during early growth. The authors found that *Calotropis procera* gave the best results as a host plant, followed by *Cassia siamea, C. calothyrsus* and *Azadirachta indica*. All of these associations resulted in significant increases in plant height and volume of the main stem of sandalwood. That *C. calothyrsus* is a suitable host for sandalwood was also confirmed in Indonesian plantations by Mindawati (1987) who found significantly better growth with this species than with three other tree species.

Whilst the seedling establishment of high-value timber tree species requires early shade, later plant development is retarded by the presence of a dense canopy in the chosen nurse species. Moreover, where nurse or host trees are marketable timbers, their felling and extraction can damage the developing timber species. *C. calothyrsus* grows rapidly and provides a host root system (as well as shade) for sandalwood seedlings without inhibiting later growth. It can also be managed through pruning without damaging the valuable sandalwood crop and may be spaced at 4 x 2 m to allow some shade, but avoid a dense closed canopy (Schinde *et al.*, 1993). Once the sandalwood is established, *C. calothyrsus* may be managed for the production of fuelwood, or fodder, provided pruning is not too intensive.

In an experiment in Rwanda, *C. calothyrsus* has been used as a support for beans. The *Phaseolus* plants benefited from both the shade of the tree, and the support they afforded (Graf, 1987). In this system, *C. calothyrsus* may be planted at densities of between 1 x 1 m and 1.5 x 1.5 m to allow the production of green manure for the subsequent crop of beans (Graf, 1987). Stems can be cut at the end of the first year for use as stakes, although the wood of the species tends to be brittle. In addition, the species' habit is less straight than comparable agroforestry trees such as *Gliricidia sepium* or *Leucaena leucocephala*, so in order to improve the form of main stems for the production of stakes, it is important to plant the species at a close spacing. Hence an initial planting at 1 x 1 m may be most applicable.

5.6   Land rehabilitation

The potential of fast-growing, nitrogen-fixing legumes in land rehabilitation programmes has been addressed by a number of authors (e.g., Goeltenboth *et al.*, 1990). *C. calothyrsus* grows rapidly under appropriate conditions to form a dense canopy which can be used to shade out weed species and improve the physical and chemical properties of degraded soil. For example, in Rwanda, the use of *C. calothyrsus* was recommended to shade out grass species, to reduce weed cover in drainage ditches, and to provide green manure for soil improvement (Egger, 1986). In the Philippines, hedgerows and contour ditches have been used to reduce erosion and increase the supply of fodder and green manure (Gradwohl and Greenberg, 1988). *C. calothyrsus* has been among a number of species suggested for fallow regeneration plantings in west Africa (Catinot, 1984), and on Réunion Island *C. calothyrsus* hedges have been used to improve soil physical properties (Cattet, 1996; Tassin *et al.,* 1996).

Calliandra calothyrsus has formed a prominent part of the Indonesian Government's re-greening efforts. Danaatmadja (1990) documents the reforestation efforts which have been undertaken on the Kaledong and Haruman mountains of west Java, in which *C. calothyrsus* was used in mixed plantations to sustain soil and water conservation in the proposed catchment area of the Jategede Reservoir. The species can also be established through aerial sowing for the purpose of land rehabilitation. In central Java, for example, aerial sowing on burnt and ploughed strips gave good results (Soemarna and Sudiono, 1974). In east Java, aerial seeding of *C. calothyrsus* was examined after one year and it was found that the cover was almost that of the minimum adequate stocking (Soemarna and Sudiono, 1974).

5.7   Honey and shellac production

Honey production

The flowers of *C. calothyrsus* are visited by bees, which are attracted to the residual nectar left after nocturnal flowering. Bees do not pollinate *C. calothyrsus*, since they do not come into contact with the anthers or stigma, but instead 'rob' nectar by clinging to the side of the floral cup. The Indonesian state forestry organisation, Perum Perhutani, has established several areas of *C. calothyrsus* within degraded forest land in order to provide a source of nectar for bees, and has initiated a programme of honey production and marketing.

In areas of Java with well-established *C. calothyrsus* trees, between 1-3 kg/colony/month of honey has been harvested from hives containing the bee *Apis cerana* (Crane *et al.*, 1984). Perum Perhutani (1990) have published figures for the area of state forest land planted with *C. calothyrsus* for honey production which, in 1989, totalled 1625 ha in the Sukabumi Forest District in west Java alone. Poedianto (1980) described the planting of *C. calothyrsus* at Gunung Arca, which comprises 601 ha within the Sukabumi Forest District. This area could potentially support 1800 hives, each of which could produce 15 kg of honey per colony per year, amounting to a potential total of 27000 kg/yr. Nadiar (1979) recorded the actual number of hives to be 600 producing a total of 10080 kg/yr. The total cost of production per kilogram was estimated at Rp. 1742 and the sale price of honey was estimated at Rp. 3000, indicating the commercial viability of the exercise, provided that honey production was seen as a by-product of forest plantation and not its justification. In addition, the presence of hives may also increase pollination events in adjacent agricultural crops.

Where *C. calothyrsus* has been planted for honey production it tends to be the dominant species, although the presence of other plants is important for the provision of pollen as a bee forage (Poedianto, 1980). The species has been planted at a high density spacing in many areas of Indonesia (1 x 1 m to 2 x 2 m), within which hives have been distributed at 10 m intervals (Nadiar, 1979). Supplementary benefits of the honey production areas have been listed as the reforestation of critical areas (particularly to protect important watersheds), the supply of fuelwood and the development of improved relations between villagers and the state forestry service (Poedianto, 1980).

Shellac production

In a number of trials, *C. calothyrsus* has also proved to be a suitable host plant for the insect *Laccifer lacca* (lac) which secretes a tough resin (shellac) that forms the basis of lacquers, dyes and polishes (Satjapradja and Sukandi,

1981). In trials, the species was grown to an age of 3-4 years, stems were cut at a height of 25 cm above the soil and seeded with lac insects after five months (Kasmudjo, 1978). Infection of the cuttings took up to seven days and the culture expanded over a three-month period, after which the host plants began to show signs of withering. Approximately 1400 g of the resin-covered insects were produced over the infection period, a quantity which was equivalent to three times as much lac as was originally used to start the culture (Kasmudjo, 1978).

5.8   General management principles

Spacing

The spacing at which *C. calothyrsus* is planted will depend on the purpose for which the species is being used, and on the chosen site. The spacing requirements for the major uses of *C. calothyrsus* are discussed in sections 5.2 to 5.7, but a summary of the spacings used in various production systems as published in the literature can be found in Table 5.3.

For the production of leaf material for animal fodder, planting arrangements, which maximise biomass production, may be compatible with the land use pattern of an existing farming system. In Africa, Paterson *et al.* (1996) observed that areas acceptable to farmers for fodder production included: boundary planting below upper storey trees, hedges around farm compounds, hedges along terrace edges on sloping land, on permanent contour bunds, and inter-cropping with grasses. All but the last option involves the use of single (or up to four) closely spaced rows of trees. The authors calculated that an average farm in the Embu region of Kenya would have a perimeter of 650 m with additional terrace hedges of up to 350 m, providing sufficient *C. calothyrsus* to produce several tonnes of fodder per year, and enough supplementary feed for all its livestock.

Baggio and Heuveldop (1984) demonstrated that spacing within the row significantly altered biomass production from the hedgerow. Spacings of 0.25-0.5 m were more productive than wider spacings of 1-2 m over a ten-month period, although the authors acknowledged that between-tree competition would increase at the narrower spacings over time. The spacing between rows of adjacent hedges varies widely, depending on the environment in which the species is grown. In Nigeria, Gichuru and Kang (1989) recommended a between row spacing of 4 m.

Table 5.3:   Spacing distances used in various agroforestry systems that have utilised *Calliandra calothyrsus*

Primary objective of planting		Spacing (m)	Reference
Leaf production	Fodder hedgerows	0.25-0.5 within rows	Baggio & Heuveldop (1984)
		0.5 within rows	Paterson *et al.* (1996)
		1 x 5	Berhe & Mohamed-Saleem (1996)
	Fodder banks	0.5 x 0.5	Ella & Blair (1989)
		0.5 x 1.5	Paterson *et al.* (1996)
		2 x 2	Berhe & Mohamed-Saleem (1996)
	Line planting in hedges	0.5 x 6	Schroth & Lehmann (1995)
		0.5 x 6	Brook (1992), Rosecrance *et al.* (1992)
		1 x 4	Gichuru & Kang (1989)
		0.5 x 3	Kartasubrata (1996)
		0.25-0.5 within rows	Akyeampong (1996)
Fuelwood	Blocks at close spacing	1 x 1	NRC (1983), CATIE (1986), Kartasubrata (1996)
		0.5 x 0.5 - 1 x 1	Ella & Blair (1989)
Poles	Blocks at wide spacing	1 x 2	NRC (1983)
		2 x 2	CATIE (1986)
Shade, supplementary production and support	Blocks at wide spacing	Coffee - 5 x 5	CATIE (1986)
		Agathis - 3 x 2	Karjono & Riyanto (1979)
		Sandalwood - 4 x 2	Schinde *et al.* (1993)
		Banana - 4 x 8	Akyeampong *et al.* (1995)
	Line planting	Beans - 1.5 x 1.5 - 5 x 5	Graf (1987)
Erosion control	Contour hedges	0.2-0.5 within rows	Tassin *et al.* (1996)
		0.25-0.5 within rows	Wiersum & Rika (1992)
Honey production	Nectar resources	Dense to 1.5 x 1.5	Poedianto (1980)

The Sloping Agricultural Land Technology (SALT) developed in the Philippines recommends a similar between-row spacing (4-5 m; Laquihon and Pagbilao, 1994). In Togo, Schroth and Lehmann (1995) found that to compensate for the reduced cropping area and competition for resources, hedges should be spaced at distances greater than 6 m.

In cases where leaf material is produced from pure plots, Ella and Blair (1989) and Ella *et al.* (1991) documented increasing leaf biomass production at decreasing spacing distances from 4 x 4 m to 0.5 x 0.5 m over 20 months. This suggests a smaller distance between trees is the preferred option in this system.

For fuelwood production, spacing distances vary from 1 x 1 m to 2 x 2 m (Ella and Blair, 1989; Kartasubrata, 1996; Wiersum and Rika, 1992). Yantasath *et al.* (1985) observed, however, that the largest dimension wood was produced at greater spacing distances (up to 1 x 4 m).

When planted with perennial crops for shade or support, the spacing of *C. calothyrsus* will depend on the associated crop. Spacing distances from 2 x 3 m to 5 x 5 m have been recommended (Akyeampong *et al.*, 1995; CATIE, 1986; Graf, 1987; Karjono and Riyanto, 1979; Schinde *et al.*, 1993). For the production of honey *C. calothyrsus* should be interspersed at regular intervals with other plant species providing sources of pollen (e.g., 10 m; Poedianto, 1980).

Weediness

When an exotic, fast-growing, agroforestry tree is introduced into a suitable environment where it is free from its natural pests and diseases, there is a risk that it may spread rapidly to the detriment of existing land use systems, or the native flora. This risk is exacerbated when the species sets seed profusely from an early age in an area where demand for the species is low. Lowry and Macklin (1988) observed that there was a possibility that *C. calothyrsus* could become weedy.

Macqueen (1992) in assessing the species ecology and biology suggested that the species would only be likely to spread rapidly in areas of recurrent disturbance. Low seed set per tree in comparison with other agroforestry species reduces the risk that the species will become a serious weed. Chamberlain and Pottinger (1995) noted that the species had colonised disturbed habitats in Uganda and Hawaii, but that the implications for land management and the native flora were unclear. In Fiji, intensive agricultural practices, including the clearing of weeds, reduced the threat. General recommendations include the need to monitor any first-time introductions with great care, and to give preference to native species where no clear advantage is apparent from the introduction of an exotic species.
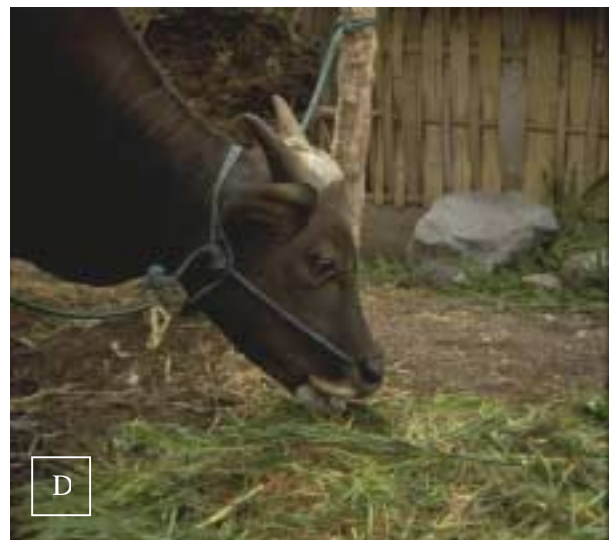
Plate 4

A - Small dimension fuelwood harvested from *Calliandra calothyrsus* in Java, Indonesia.

B - Large dimension wood for poles or fuelwood harvested from *Calliandra calothyrsus* in Java, Indonesia.

C - Honey production in a naturalised population of *Calliandra calothyrsus*, Java, Indonesia.

D - Leaves of *Calliandra calothyrsus* being used as cattle fodder in Sumatra, Indonesia.

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6   Provenance Trials

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6.1   Introduction

The introduction of a tree species into a country is rarely carried out in a planned and systematic manner. Ideally, the full range of genetic variability should be evaluated for productivity and assessed for environmental impact prior to release of seed or vegetative material for planting programmes. In practice, however, most tree introductions take place in an *ad hoc* manner, sometimes through the interests of plant collectors, often by travellers or colonists bringing with them trees which they know to be useful in their homelands, and sometimes by foresters wishing to experiment with new species. Whatever the means of introduction, the eagerness to investigate new species coupled with a lack of appreciation of the genetic diversity contained within most species generally means that introductions are made without any prior formal evaluation and are usually of narrow genetic base (Hughes, 1994). Although introduced species frequently grow well in comparison with native species, it is the experience of foresters in both tropical and temperate countries that the initial source of seed used for introductions is rarely the most productive (Zobel and Talbert, 1984; Barnes and Simons, 1994).

Provenance trials enable a range of seed sources of a single species to be evaluated on a given site and provide evidence upon which to base future decisions surrounding seed procurement. However, they are frequently overlooked in introduction programmes partly due to the expense and practical difficulty in obtaining a wide range of seed sources, but also often due to a lack of understanding of their fundamental importance to future planting decisions. Large gains in productivity can be made through the selection of the best provenance of a species for a given site and purpose.

For *C. calothyrsus*, along with many other agroforestry tree species, the initial interest in the performance of the species developed from a limited selection of the genetic diversity contained within the species (Pottinger, 1996b). *C. calothyrsus* is planted widely in Indonesia, and until recently almost all seed available to researchers and those interested in planting programmes, came from this source. This 'land race' was itself derived from seed originating from a small area of Guatemala (see Chapter 7). In view of the growing international interest in *C. calothyrsus* for use on farms, combined with the lack of knowledge surrounding its genetic resources, a programme was initiated in 1990 at the Oxford Forestry Institute (OFI) to carry out a comprehensive exploration, collection and evaluation of *C. calothyrsus* and its close relatives (Macqueen 1991; Pottinger 1996b).

Investigation of the genetic resource in its native range

Between 1990 and 1994 an intensive investigation of the genetic resources *of C. calothyrsus* was undertaken throughout its native range. This involved mapping the complete distribution of the species, including previously unrecorded populations, the establishment of provenance boundaries, collection of seed from throughout the native range and a detailed review and re-classification of the taxonomy within the series *Racemosae* (Macqueen 1992; Macqueen and Hernández, 1997). Fifty provenances were recorded and over 50 kg of seed were eventually collected from eight countries. Provenance details and collection sites are listed in Table 6.1.

Within each provenance, seed was collected from a minimum of 25 trees spaced at least 50m apart with no phenotypic selection criteria employed in order to provide as broad a representation as possible of the genetic variation present (Macqueen 1993a). Most seed was bulked following collection but in a few cases where significant amounts of seed were available from a range of trees, individual collections were kept separate to accommodate the potential of carrying out family selection for later breeding efforts.

Table 6.1:   Provenances of *Calliandra calothyrsus* included in the trials evaluated in the International *Calliandra* Trial Network.

Provenance	Country	OFI Identification No.	Altitude (m)	Rainfall (mm)
Georgesville	Belize	21/91, 48/92	350	1539
Gracie Rock	Belize	46/92	90	2313
Santa Cruz	Belize	22/91, 45/92	150	3068
Agua Zarcas	Costa Rica	59/93	200	2770
Fortuna	Costa Rica	18/91, 56/93, 108/94	85	4718
Los Chiles	Costa Rica	53/93	60	1944
San Isidro del General	Costa Rica	57/93	700	2951
San Miguel	Costa Rica	60/93	1300	1867
Santa María	Costa Rica	19/91	825	3222
Turrialba	Costa Rica	20/91, 54/93	800	2363
Upala	Costa Rica	55/93	100	2589
Alotenango	Guatemala	16/91, 47/92	1100	1203
Barillas	Guatemala	51/92, 35/93	1320	5829
Cobán	Guatemala	8/91	1300	2517
Flores	Guatemala	10/91	220	1994
Patulul	Guatemala	9/91, 51/92, 153/92, 34/93	330	3185
Santa María de Jesus	Guatemala	53/92, 33/93	1500	4236
Cofradia	Honduras	14/91	300	1091
Gualaco	Honduras	48/93	610	905
La Ceiba	Honduras	12/91, 17/91, 73/92, 15/96	80	2884
Lago Yojoa	Honduras	46/93	550	2596
Las Flores	Honduras	23/91, 75/93	1076	1688
San Esteban	Honduras	47/93	420	3318
Santa María	Honduras	13/91	500	1145
Trujillo	Honduras	15/91, 49/93	50	2715
Bandung	Indonesia	148/91	715	1949
Maduin	Indonesia	147/91	800	1884
Apic Apac	Mexico	60/92	860	898
Bombana	Mexico	61/92, 31/93	950	1256
Bonampak	Mexico	62/92	400	2156
Chilon	Mexico	49/92, 37/93	769	1515
Ixtapa	Mexico	40/92	1100	1701
Ococingo	Mexico	36/93	900-1500	1804
Plan del Rio	Mexico	44/92	240	1957
Union Juarez	Mexico	50/92	930	3786
La Puerta	Nicaragua	134/91, 109/94	600	1889
San Ramón	Nicaragua	11/91, 110/94	850	1394
Boquete	Panama	62/93	1200	3735
Cangandi	Panama	63/93	10	3036

6.2 International *Calliandra* Provenance Trial Network

Seed of *C. calothyrsus* collected between 1990 and 1994 was sent initially to the UK for temporary storage from where it was subsequently distributed to 48 organisations in 39 countries for the establishment of field trials to investigate the performance of the provenances. Two experimental designs were proposed to evaluate the performance of different provenances when grown principally for leaf production or fuelwood. However, in order to accommodate the many varied planting designs used on small farms, some degree of flexibility was permitted in spacing and cutting regimes.

Practical limitations to the collection of seed and trial management meant that only a proportion of the 50 provenances collected were sent to each collaborator. In addition, poor germination was encountered at a number sites, which also resulted in an unequal provenance representation across the trials.

Information provided by collaborators

Trial results were received from a number of collaborators, and this data formed the basis of the evaluation of the International *Calliandra* Trial Network. The evaluation was restricted to 37 provenances of *C. calothyrsus* (see Table 6.1). Altogether, data from 21 trials were included in the analysis, and a full list of these trials is given in Table 6.2. Provenance representation was unequal for the reasons described above, with none of the provenances planted on all the sites and several provenances represented in just one trial.

Evaluation methodology

Since stem length was the only trait assessed in a standard manner, it was used as a basic indicator of growth, although it is acknowledged that it is almost never a trait of any importance to growers of *C. calothyrsus*. Wood production and leaf production, the traits of interest to most growers, were assessed on most sites. The assessment methods, however, differed widely and were not always stated with the results. This meant that a combined analysis of all 21 trials was impossible. Direct comparisons across all sites for these traits were, therefore, difficult to make and the conclusions should be interpreted with caution.

To overcome the differences in assessment methodologies, a method of standardisation was needed in the expression of provenance performance on all the sites. The simplest such method is to use the site mean as a benchmark, and express provenance performance relative to this. The unequal representation of provenances in the trials, however, made this impossible, because each site mean would be biased by the set of provenances represented on that site. An alternative method is to use a control seedlot, or to form a benchmark using a subset of provenances. In this analysis, three provenances were represented on 19 sites, and the mean of these three provenances was used as the benchmark value on each site. Evaluation of provenance performance, relative to this benchmark was restricted to categorising performance into four classes: above all three provenances used in forming the benchmark; above the benchmark value; below the benchmark value; and below all three provenances. The frequency with which a provenance falls into each of these categories gives a robust guide to the stability of the provenance's performance across the sites, an important consideration when making recommendations about choosing provenances for a wide range of conditions.

Finally, since farmers are primarily interested simply in those provenances which produce the most wood or leaf material, a study was made of the frequency with which each provenance was one of the top three performing provenances on each site. Whilst this frequency is obviously affected by the number of sites the provenances are represented on and the number of provenances on each site, it gives a simple and quick guide to promising provenances.

Trial results

Stem length

Table 6.3 shows the mean stem length for each provenance at each site on which it was planted, with the overall site mean and pooled standard deviation (where known) given as a guide to variation within the site. Figure 6.1a shows the performance of the provenances in comparison to the benchmark, broken down into the four categories described above. The performances of the three provenances used to form the benchmark are shown relative to one other. Figure 6.1b shows the frequency with which each provenance was one of the top three performing provenances on a site.

From Figure 6.1a it can be seen that Flores from Guatemala was generally the best of the three benchmark provenances, whilst La Ceiba from Honduras was almost always the worst of these three. Of the other provenances, it can be noted that Georgesville and Gracie Rock, both from Belize, and the Costa Rican Santa María provenance always outperformed all three of the benchmark provenances.

Table 6.2:   Trial details and site descriptions of *Calliandra calothyrsus* provenance trials.

Trial site	Trial code	Collaborating organisation	Site location	Altitude (m)	Rainfall (mm)	Soil pH
Australia						
Landsdowne	Lan. Aus		na	60	870*	5.8
Utchee Creek	Utc. Aus		na	250	3500	5.0
Cameroon						
Minkoameyos	Min. Cmr	IRA/ICRAF Agroforestry Programme	3ºN, 11ºE	813	1690	6.0
Nkoemvone	Nko. Cmr	IRA/ICRAF Agroforestry Programme	2ºN, 12ºE	630	1820	4.5
Yaounde	Yao. Cmr	IRA/ICRAF Agroforestry Programme	3ºN, 11ºE	813	1690	6.0
Colombia						
Santander de Quilichao	Quil. Col	Centro de Investigacíon Agricola Tropical	3ºS, 76ºW	1000	1800	4.0
Ethiopia						
Bako	Bak. Eth	Institute of Agricultural Research	6ºN, 37ºE	1000	1500	6.0
Fiji						
Nadruloulou	Nad. Fij	Fiji-German Forestry Project	17ºS, 174ºE	30	3410	na
Jamaica						
Cinchona	Cin. Jam	University of West Indies	18ºN, 76ºW	1550	2500	5.4
Kenya						
Embu	Emb. Ken	ICRAF	1ºS, 37ºE	1480	1200	na
Madagascar						
Antananarivo	Ant. Mdg	National Seed Bank	na	na	na	na
Mexico						
Yapacani	Yap. Mex	Centro de Investigacíon Agricola Tropical	na	945	1800	5.7
New Caledonia						
Port Laguerre	Lag. Nca	CIRAD Forêt	22ºS, 165ºE	300	1653	Acidic
Philippines						
Los Banos	Ban. Phl	Ecosystems Research & Development Bureau	14ºN, 121ºE	400	2200	4.6
Sri Lanka						
Doragala	Dor. Srl	GTZ	6ºN, 81ºE	426	1050	5.3
Pallekelle	Pal. Srl	GTZ	6ºN, 81ºE	1450	1500	4.5
Tanzania						
SUA farm	SFm. Tan	Sokoine University of Agriculture	6ºS, 37ºE	526	800	6.5
Gairo	Gai. Tan	Sokoine University of Agriculture	6ºS, 36ºE	1200	500	6.0
Uganda						
Kifu	Kif. Uga	ICRAF	2ºN, 34ºE	2000	1040	4.6
Zambia						
Misamfu	Mis. Zam	ICRAF	10ºS, 31ºE	1300	1360	Acidic
Zimbabwe						
Domboshawa	Dom. Zim	ICRAF	17ºS, 31ºE	1530	895	4.6

*   The Landsdowne site in Australia was trickle irrigated.
na   Data not available

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Table 6.3:   Summary of results from the *Calliandra calothyrsus* trial series: stem length (m).

Trial Site	Min. Cmr	Nko. Cmr	Yao. Cmr	Quil. Col	Cin. Jam	Ant. Mdg	Yap. Mex	Lag. Nca	Kif. Uga
Site mean	3.55	3.66	5.13	2.03	2.07	0.74	2.82	2.15	5.76
Std. dev.	-	-	-	*0.39*	*0.48*	-	*0.75*	-	*0.88*
Provenance:									
Georgesville				2.24		0.98	3.32		6.29
Gracie Rock								2.64	
Santa Cruz	3.08	2.73	5.27						
Agua Zarcas				2.14					
Fortuna	3.59	4.52	4.54	1.86		0.75		2.38	6.05
San Miguel							2.51		
Santa María (CR)				2.18					
Turrialba	3.54	3.14	5.27				2.89	1.96	
Upala				2.00					
Barillas	3.72	3.80	5.75			0.69	2.98		
Cobán	3.87	4.05	5.50				2.68		5.80
Flores	4.35	4.26	5.50	2.16	2.18	0.69	3.04	2.58	6.16
Patulul				2.20	2.54	0.65	3.24		
Santa María de Jesus	3.15	3.61	4.88	2.17	2.31	0.78	2.60	2.05	5.39
Gualaco								2.19	
La Ceiba	2.71	2.96	4.24	1.43	1.46	0.70	2.44	1.86	4.70
Lago Yojoa					1.83				
Las Flores							2.22		
San Esteban								1.82	5.14
Santa María (H)	3.14	3.28	4.82						
Trujillo			4.62		1.84				
Maduin	3.39	3.82	5.43	1.96		0.65		2.14	6.30
Apic Apac									
Bombana	3.92	4.21	5.01				3.12	2.67	6.42
Bonampak	4.15	3.85	5.80		2.34	0.74	3.18		
Chilon				2.05			2.62		
Ixtapa								1.39	
La Puerta	3.45	3.34	5.13			0.77		2.17	
San Ramón	3.64	3.70	5.17	2.02		0.79	2.77	2.04	5.02
Boquete				2.00					

Figure 6.1a: Performance of *Calliandra calothyrsus* provenances relative to 'benchmark' – stem length. The provenances used as a benchmark were Flores, Santa María de Jesus and La Ceiba.

Figure 6.1b: Top performing provenances of *Calliandra calothyrsus* – stem length. Number of trials in which each provenance was assessed are shown in parentheses.
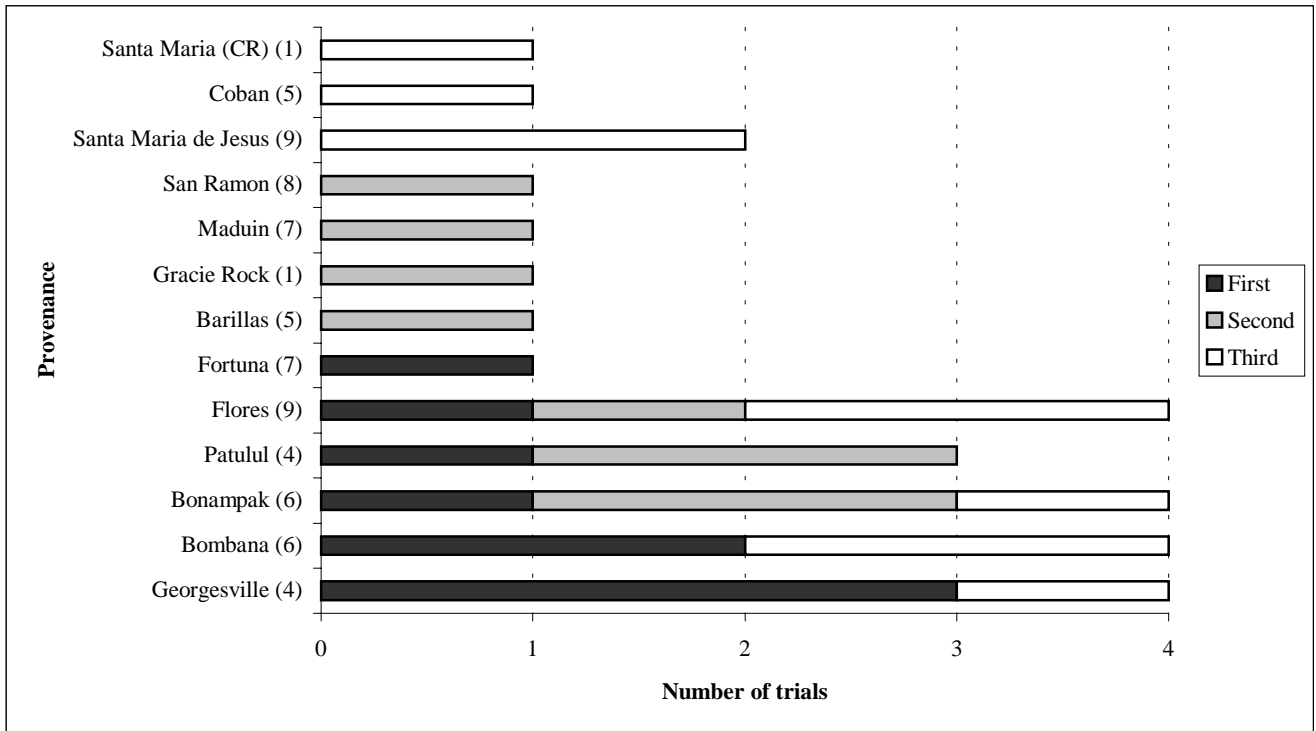
This is more significant in the case of Georgesville, since it was represented on four sites whereas the other two provenances appeared on only one site. All the Guatemalan provenances had above-average stem length, an interesting result that corresponds with results found in a study of *Gliricidia sepium* provenances (Dunsdon and Simons, 1996). The Honduran provenances were all below average for this trait. Figure 6.1b shows Georgesville to be among the top three performing provenances on all four sites at which it was represented and the top performer on three of those sites.

Wood production

Table 6.4 shows the mean wood production for each provenance at each site on which it was planted. The figures shown are a mixture of fresh weights and dry weights, expressed on a kilogram/tree or tonne/hectare basis. The overall site mean and pooled standard deviation are given where known. Figure 6.2a shows the wood production of each provenance relative to the benchmark, broken down into the four categories described earlier. The performances of the three provenances used to form the benchmark are shown relative to each other. Figure 6.2b shows the frequency with which each provenance was one of the top three

performing provenances on a site.

Figure 6.2a clearly illustrates that there is much greater variation in provenance performance for wood production than for stem length. This is to be expected, and is in part due to the responses of the provenances to different management regimes and assessment methodologies. This greater variation in provenance performance means that the provenances that always had above-average wood production were those that were only represented on a small number of sites, Gracie Rock and Bandung being the only such provenances that were at more than one site. La Puerta, Georgesville, San Ramón and Barillas were all generally high wood producers. Once again the Guatemalan provenances generally performed well, as did the Nicaraguan provenances for wood production. It is also worth noting the above-average performance of the two Indonesian land-race seedlots. This is confirmed, in Figure 6.2b, by the high frequency with which Maduin in particular was one of the top three performing provenances, bettered only by San Ramón.

Table 6.4: Summary of results from the *Calliandra calothyrsus* trial series: wood production.

Trial site	Lan. Aus	Utc. Aus	Yao. Cmr	Bak. Eth	Nad. Fij	Cin. Jam	Emb. Ken	Ban. Phl	Dor. Srl	Pal. Srl	SFm. Tan	Gai. Tan	Kif. Uga	Mis. Zam
Site mean	4.07	1.55	29.5	0.89	2.43	33.3	3.31	0.82	1.75	1.25	7.35	9.43	28.5	2.18
Std. dev.	*0.98*	*0.51*	*-*	*0.42*	*-*	*22.2*	*-*	*0.82*	*-*	*-*	*-*	*-*	*12.5*	*-*
Provenance:														
Georgesville	5.68	1.55						0.73	1.84	1.05			24.6	
Gracie Rock				1.09	3.61									2.24
Santa Cruz			29.1								8.13			
Agua Zarcas				0.79										
Fortuna	3.38	1.37	31.2		2.79		3.04		2.06	1.03	6.51	14.73	23.2	
Los Chiles								0.85						
San Miguel											4.99	12.77		3.04
Turrialba	3.89	1.27	31.9	0.88					1.80	1.21				
Alotenango											7.15	10.50		
Barillas	3.74	1.74	33.9		2.56			0.93	1.59	1.49				2.23
Cobán	4.13	1.34	23.1	0.87			3.06		1.53	1.21			27.5	
Flores	4.59	1.73	32.5	0.74	1.77	43.1	2.44		1.28	1.30	9.72		32.8	1.70
Patulul	5.17	1.49				57.8			1.68	1.16	3.90			
S M de Jesus	4.13	2.03	21.7	0.97	1.21	37.6	4.44		1.63	1.24	5.60		24.8	1.68
Cofradia												9.16		0.94
La Ceiba	4.21	1.26	19.7	0.73	3.65	27.0	2.66		1.66		6.86		22.8	1.49
Lago Yojoa						10.9								
Las Flores	2.99	0.89												
San Esteban									1.64	1.29			28.0	
Santa María	3.30	1.49	25.1											
Trujillo			21.7			35.9								
Bandung											8.74	11.67		3.59
Maduin	3.77	2.21	35.3		1.40		3.68		1.88	1.28			34.8	1.32
Apic Apac												6.37		
Bombana			32.5	0.75	2.09				1.94	1.16	7.96	5.99	27.5	3.19
Bonampak			31.9			35.4								
Ixtapa	2.87	1.09		0.92							4.86			2.49
Ococingo								1.01						
Plan del Rio	3.71	1.03							2.51	1.53	7.83	7.81		1.70
Union Juarez	4.65	1.51							1.24	1.55	9.03			3.32
La Puerta	4.99	2.35	33.9											1.55
San Ramón	4.03	1.96	39.3	1.15	2.89		3.88	0.59	2.02	1.06	11.67	5.83	32.2	2.19
Cangandi					2.33									

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Figure 6.2a: Performance of *Calliandra calothyrsus* provenances relative to 'benchmark' – wood production. The provenances used as a benchmark were Flores, Santa María de Jesus and La Ceiba.
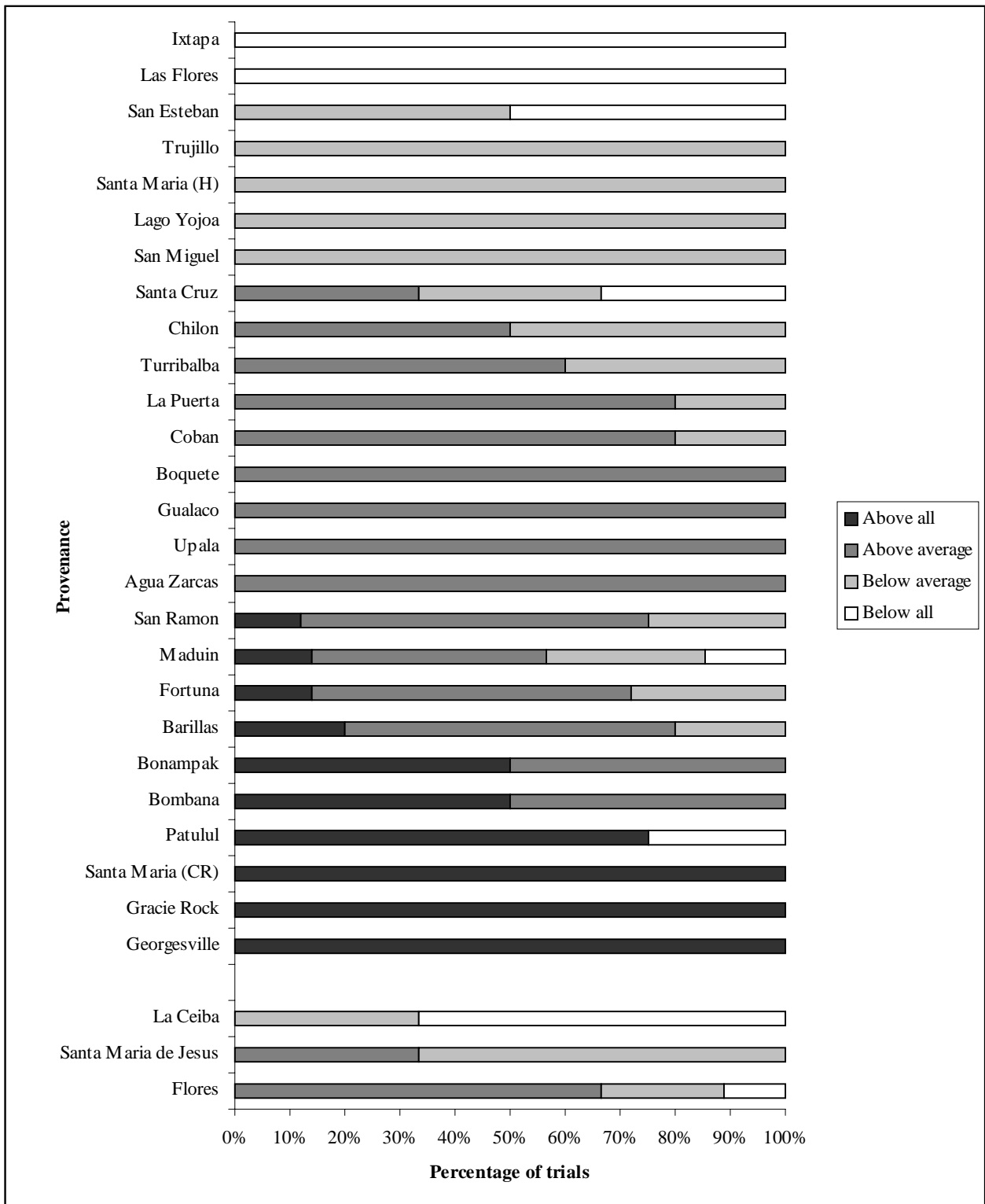
Figure 6.2b: Top performing provenances of *Calliandra calothyrsus* – wood production. Number of trials in which each provenance was assessed are shown in parentheses.
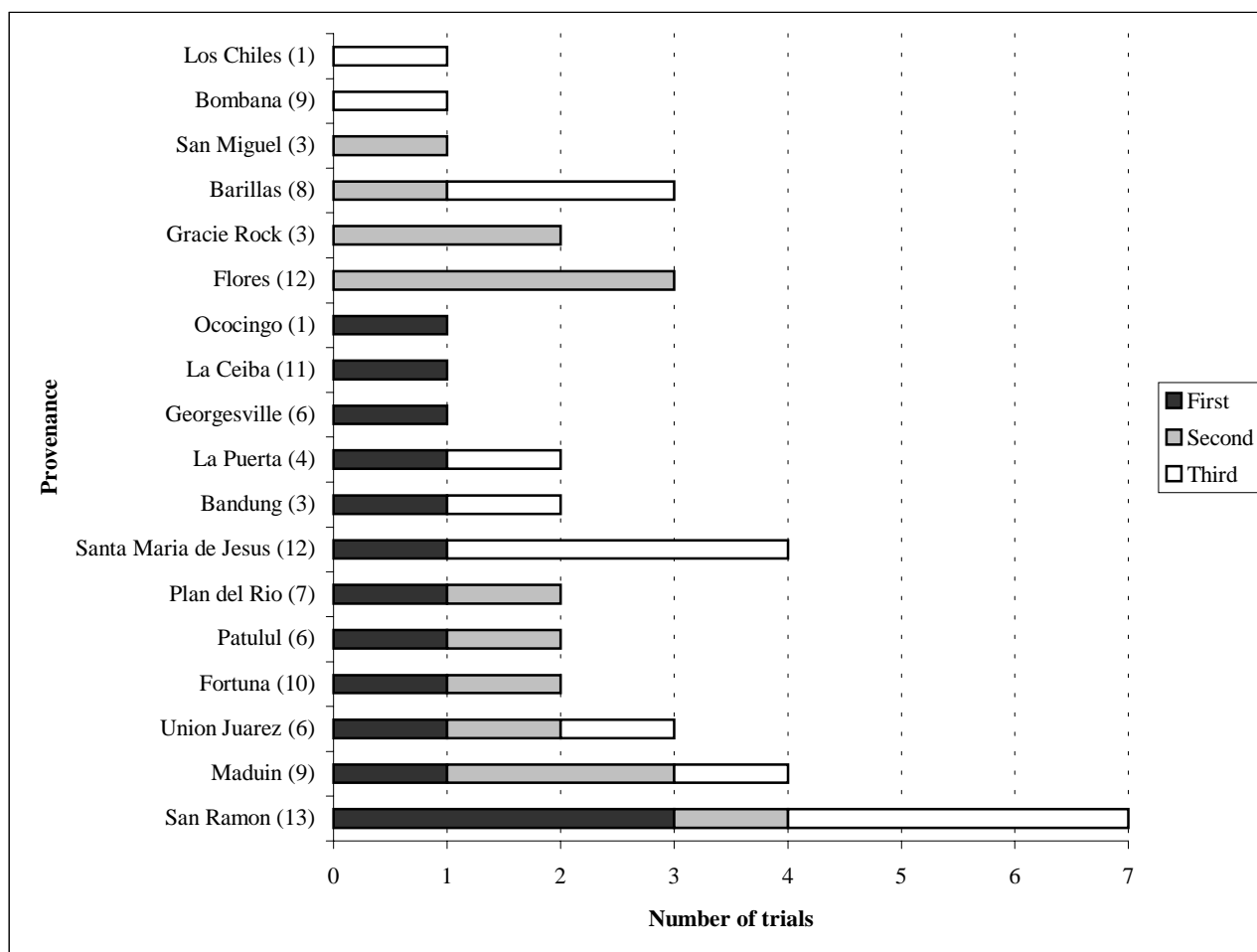
Leaf production

Table 6.5 shows the mean leaf production for each provenance at each site on which it was planted. The figures shown are again a mixture of fresh weights and dry weights, expressed on a kilogram/tree or tonne/hectare basis. The overall site mean and pooled standard deviation are given where known. Figure 6.3a shows the leaf production of each provenance relative to the benchmark, broken down into the four categories described earlier. The performances of the three provenances used to form the benchmark are shown relative to each other. Figure 6.3b shows the frequency with which each provenance was one of the top three performing provenances on a site.

It was expected that leaf production would show still greater variation in provenance performance than the other two traits, but this appears not to be the case. Comparing Figure 6.3a with Figure 6.2a shows that, on

the contrary, there appears to be less variation in provenance performance for leaf production than for wood production. From Figure 6.3a, the performance of San Ramón is particularly notable. This provenance's performance was above average in terms of leaf production on every one of the 13 sites at which it was represented and outperformed all three of the benchmark provenances on nine of those sites. Figure 6.3b confirms this, showing San Ramón to be the top performing provenance on six sites and one of the top three performers on ten sites. With no below-average performances, San Ramón is clearly the 'best-bet' provenance for leaf production. As with wood production, the two Indonesian land-race seedlots are generally above-average performers, and the Nicaraguan provenances are generally above average. The performance of the Guatemalan provenances is more mixed for this trait.

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Table 6.5:   Summary of results from the *Calliandra calothyrsus* trial series: leaf production.

Trial site	Lan. Aus	Utc. Aus	Min. Cmr	Nko. Cmr	Yao. Cmr	Bak. Eth	Nad. Fij	Cin. Jam	Emb. Ken	Ban. Phl	Dor. Srl	Pal. Srl	Sfm. Tan	Gai. Tan	Kif. Uga	Mis. Zam	Dom. Zim
Site mean	3.96	2.35	1.62	1.29	5.19	2.01	1.33	52.5	5.85	0.57	2.68	2.68	3.52	4.39	8.20	0.64	3.28
Std. dev.	*0.86*	*0.67*	-	-	-	*0.31*	-	*34.2*	-	*0.42*	-	-	-	-	*4.46*	-	-
Provenance:																	
Georgesville	4.92	2.58								0.50	2.85	2.39			6.54		
Gracie Rock						2.20	1.69									0.56	
Santa Cruz			1.62	0.77	5.15								3.91				2.70
Agua Zarcas						1.81											
Fortuna	3.01	1.84	1.61	1.88	4.75		1.29		5.49		2.94	2.31	3.10	6.52	6.33		3.70
Los Chiles										0.65							
S I del General																	3.70
San Miguel													2.19	5.89		0.75	
Turrialba	3.19	1.80	1.95	1.11	5.15	2.18					2.80	2.63					
Alotenango													3.35	4.91			
Barillas	3.65	2.32	1.50	1.16	5.56		1.44			0.64	2.48	2.87				0.63	
Cobán	3.68	1.76	1.59	1.59	3.93	1.81			5.53		2.36	2.71			7.24		
Flores	4.94	2.41	1.85	1.41	5.49	1.66	1.07	63.1	4.69		2.08	2.57	4.57		7.40	0.47	4.30
Patulul	4.67	2.36						57.8			2.57	2.35	1.85				3.80
S M de Jesus	3.65	3.11	1.40	1.20	3.93	2.20	0.94	74.8	7.13		2.93	2.82	2.93		7.03	0.70	3.10
Cofradia														4.49		0.53	
La Ceiba	3.88	1.95	1.32	1.57	3.86	1.65	1.97	46.7	4.36		2.43		3.10		7.30	0.33	2.30
Lago Yojoa								18.0									
Las Flores	4.06	1.95															3.20
San Esteban											2.66	2.63			10.8		
Santa María	3.49	2.55	1.20	1.06	6.24												
Trujillo					3.19			57.9									
Bandung													4.27	5.18		0.71	
Maduin	3.67	3.06	1.91	1.15	5.76				5.77		2.70	2.84			7.90	0.69	2.70
Apic Apac														2.91			
Bombana			1.49	1.15	5.42	1.71	1.14				2.81	2.56	3.98		6.93	0.74	2.90
Bonampak			1.54	1.40	4.95			47.2									3.40
Ixtapa	3.39	1.87				2.21							2.44			0.63	
Ococingo										0.52							
Plan del Rio	4.08	1.97									3.52	3.02	3.76	4.03		0.57	
Union Juarez	4.26	2.43									2.20	3.34	4.44			0.61	
La Puerta	4.51	3.43	1.85	1.12	7.53											0.82	
San Ramón	4.18	2.64	1.89	1.51	6.92	2.66	2.08		7.96	0.55	2.94	2.46	5.40	2.80	12.5	0.88	3.50
Cangandi							0.91										

Figure 6.3a: Performance of *Calliandra calothyrsus* provenances relative to 'benchmark' – leaf production. The provenances used as a benchmark were Flores, Santa María de Jesus and La Ceiba.
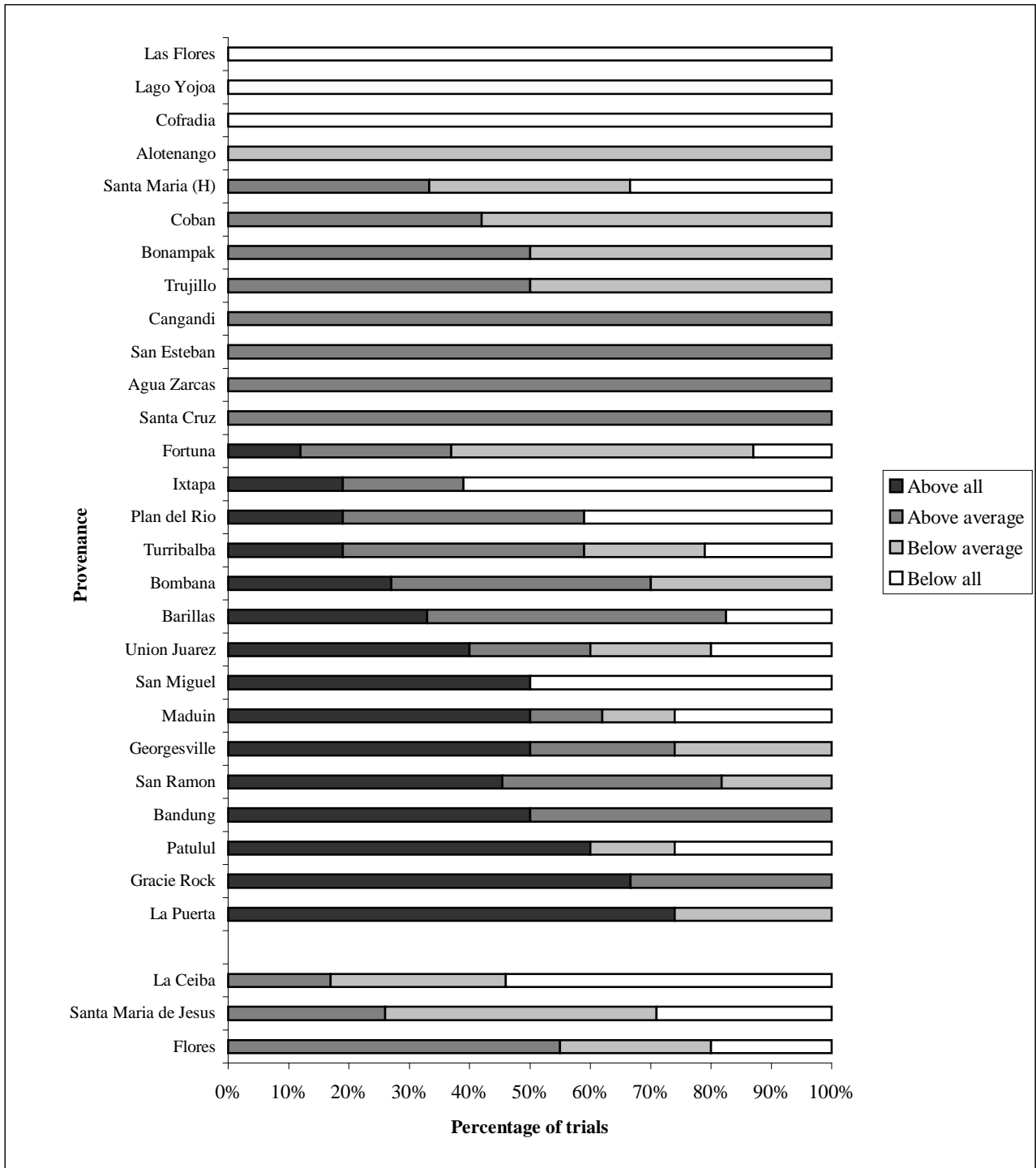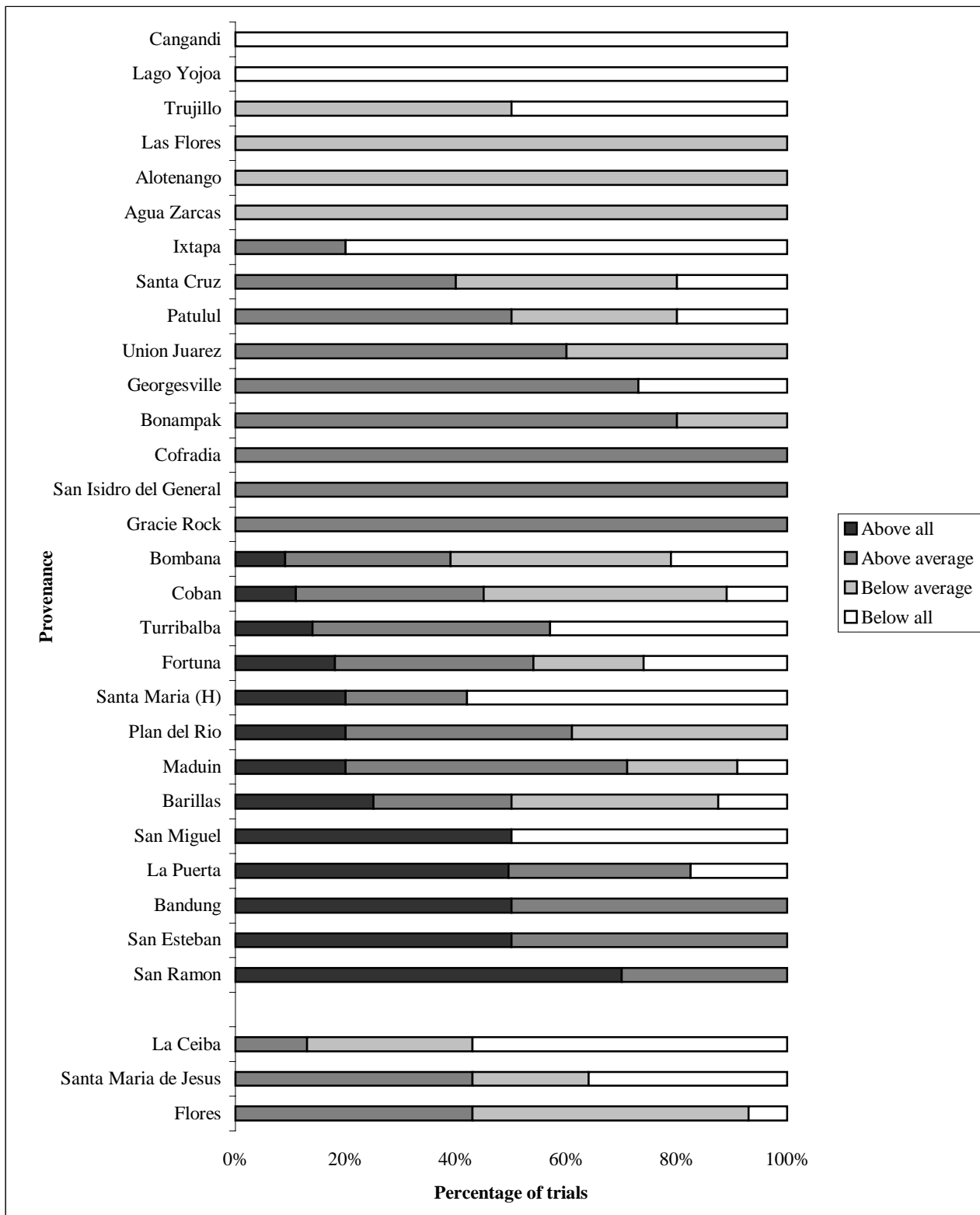
Figure 6.3b: Top performing provenances of *Calliandra calothyrsus* – leaf production. Number of trials in which each provenance was assessed are shown in parentheses.
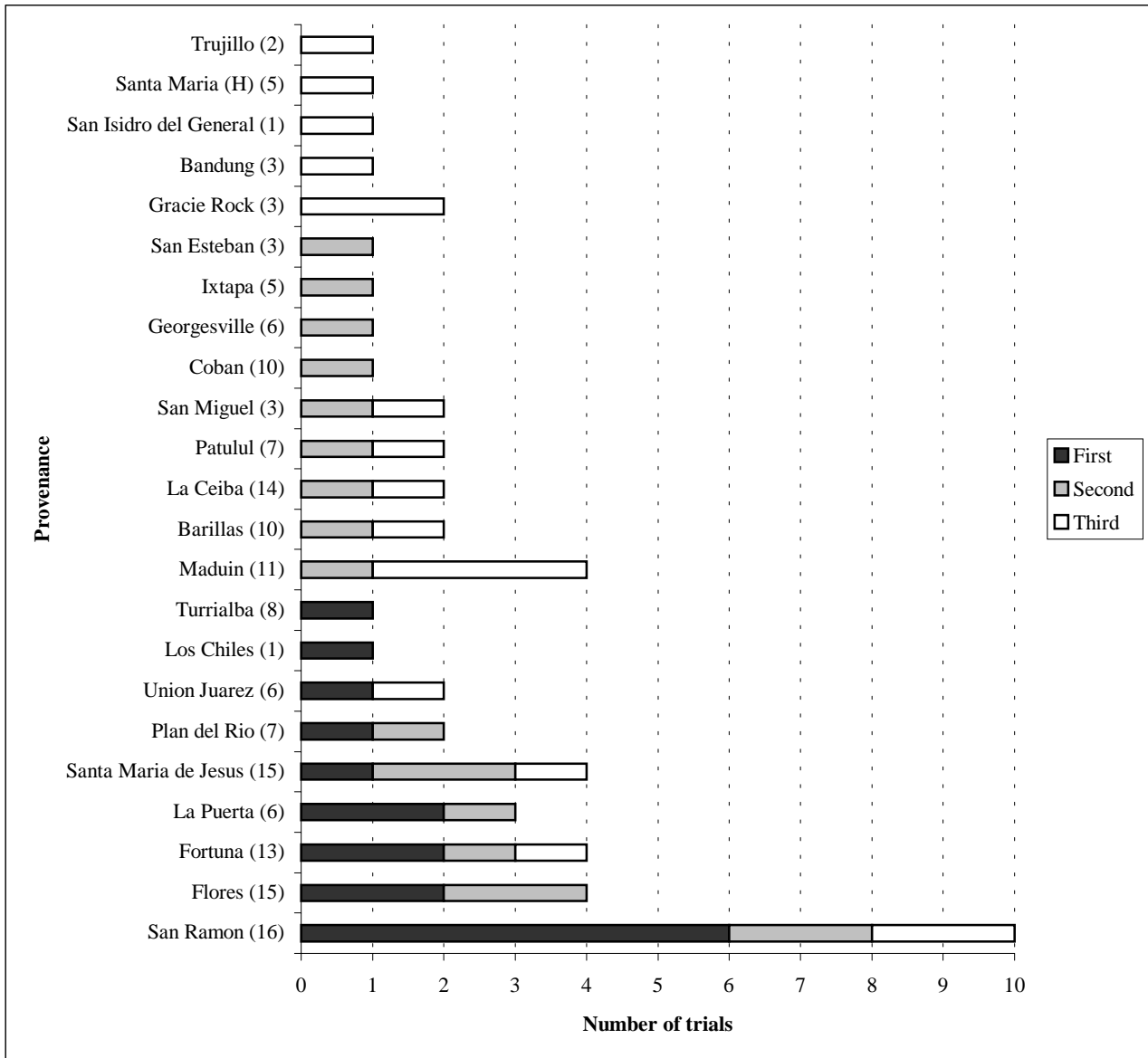
Conclusions

Despite the incomplete nature of the data set upon which this analysis was based, and the resulting difficulty in making a direct cross-site comparison, there are a number of interesting and important conclusions that can be drawn.

Since wood production and leaf production are the two traits for which *C. calothyrsus* is generally planted, there is clearly one provenance that appears to be the most promising. In terms of frequency of top performance, and stability of above-average performance, the Nicaraguan provenance of San

Ramón emerges as superior, especially strongly so for leaf production. This provenance could thus be recommended as a 'best bet' provenance for planting.

The Indonesian land-race seedlots were shown to be amongst the better performers, both in terms of leaf production and particularly wood production. These are already more widely distributed and available than the more-recently collected OFI seed sources, and it would seem that, with the exception of San Ramón, the newer seed sources have little benefit to offer over the land-race material in terms of wood and leaf production.

An interesting result was noted in the stem length analysis. This trait is often used as a basic indicator of growth as it generally less affected by management techniques (measurement takes place before lopping begins). It is therefore noteworthy that the Guatemalan provenances of *C. calothyrsus* included in this trial were all amongst the top-performing provenances for this trait as results also showed in a similar study of the genetic variation of *G. sepium.*

A.J. Pottinger and A.J. Dunsdon

Plate 5

A - Sowing *Calliandra calothyrsus* seed in the nursery, Honduras.

B – A trial plot of *Calliandra calothyrsus* planted at Lancetilla Botanic Gardens, Tela, Honduras after nine months of growth.

C - A provenance trial of *Calliandra calothyrsus* planted at ICTA, Cuyuta, Guatemala after 21 months of growth.

D - Biomass assessment of a trial plot of *Calliandra calothyrsus* planted at Lancetilla Botanic Gardens, Tela, Honduras

E - Biomass assessment of a trial plot of *Calliandra calothyrsus* planted at Dodangolla, University of Peradeniya, Sri Lanka.

F - Separation of wood and leaf biomass at the trial plot at Dodangolla, University of Peradeniya, Sri Lanka.

7   Molecular Analysis of Genetic Variation

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7.1   Introduction

Knowledge of the level, structure and origin of genetic variation within and between plant populations is important for the effective utilisation and conservation of species (Hamrick, 1992; Yeh, 1989). Factors determining the level and structure of genetic variation within plant species include evolutionary history, life-history characteristics, population density, mating system, and mechanisms of gene flow (Hamrick *et al.*, 1992; Loveless, 1992; Loveless and Hamrick, 1984; Schaal, 1980). Although morphological characters and agronomic traits have been used traditionally to characterise levels and patterns of diversity, these traits alone represent only a small portion of the plant genome and are also influenced by environmental factors, thereby limiting their utility in describing the potentially complex genetic structures which may exist within and between taxa (Avise, 1994). To overcome these constraints, various molecular approaches for the detection of variation have been devised, including the use of isozymes (Soltis and Soltis, 1990) and techniques based on the polymerase chain reaction (PCR) (Bachmann, 1994). These 'markers' of genetic variation are generally independent of environmental factors and more numerous than phenotypic characters, thereby providing a clearer indication of the underlying variation in the genome of an organism (Avise, 1994).

The purpose of this chapter is to discuss the application of molecular markers to questions regarding patterns of genetic variation within *Calliandra* series *Racemosae sensu* Macqueen and Hernández (1997) which are otherwise difficult to resolve by more conventional approaches. For those readers not familiar with the techniques of molecular analyses, the procedures employed are summarised briefly in section 7.2, while section 7.3 describes their specific application in the genus. The results are summarised in terms of the implications for: (1) optimum resource collection, evaluation and *ex situ* conservation; (2) *in situ* conservation; (3) seed orchard design; and (4) release of germplasm in exotic locations.

7.2   Molecular analyses

Isozyme analysis

Isozyme analysis relies on the movement of protein molecules through an acrylamide or starch gel medium in response to an applied electric current (electrophoresis) (Soltis and Soltis, 1990). If the protein occurs in different forms, with different amino acid compositions, then differences in the overall ionic charges of the molecules can result. This will cause variation in their electrophoretic mobilities and hence the different forms of the protein (isozymes) will migrate at different speeds through the gel medium. The isozymes are detected and visualised by chemical staining of the gel for the protein in question. Because different isozymes are inherited co-dominantly at individual loci[1], it is possible to assign a genotype to an individual from its electrophoretic profile (Avise, 1994; Brown, 1979; Weeden and Wendel, 1990).

Isozyme analysis has been applied widely to characterise patterns of genetic diversity and differentiation in plant species (Hamrick *et al.*, 1992). There are numerous reasons for this, which include the relative simplicity of the approach that allows data to be collected quickly from large sample sizes, the low cost compared to other molecular techniques, and its early recognition and availability in the history of genetic marker development (Avise, 1994). Studies with tree species are numerous and include many common agroforestry species such as *Gliricidia sepium* (Chamberlain *et al.*, 1996), *Faidherbia albida* (Harris *et al.*, 1997), *Leucaena leucocephala* (Harris *et al.*, 1994), and *Prosopis juliflora* (Solbrig and Bawa, 1975).

PCR analysis

The polymerase chain reaction (PCR analysis) is a technique that allows the selective *in vitro* amplification, or multiplication, of DNA. The logic of PCR is simple in principle, and is based on the binding of short DNA sequences (oligonucleotides) to complementary

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[1] In this case, a *locus* is a site in the genome encoding a particular protein; if a diploid individual is *heterozygous* at that locus, it possesses two different forms of the gene (*alleles*) coding for two forms of the protein (*isozymes*).

sequences in the DNA of an organism, followed by synthesis of new DNA strands primed (initiated) by these oligonucleotides and catalysed by the enzyme DNA polymerase, using deoxyribonucleotide triphosphates (dNTPs), the 'building blocks' of DNA, as substrates. DNA amplification, is brought about by repeated temperature-controlled cycling through three basic steps: (1) melting of double-stranded DNA to form two complementary single strands; (2) annealing (binding) of the oligonucleotide 'primers' to target sequences in the single-stranded DNA; and (3) extension of the primers using the target DNA as a template, thereby producing new double-stranded copies of the original DNA. Automation of the amplification process is facilitated by employing thermal cycling machines and an extremely thermostable enzyme, *Taq* polymerase, which is capable of survival at 95ºC, the temperature required to melt double-stranded DNA (Vosberg, 1989).

Based on PCR, a wide range of approaches have become available for the detection of genetic variation in plants and animals (Bachmann, 1994). Methods differ in the type of primers used for analysis, which can be either targeted to specific regions of the genome, or alternatively, chosen at random to amplify unspecified regions. Of the approaches based on unspecified targeting, random amplified polymorphic DNA (RAPD) analysis is the most common, relying on primers of arbitrary sequence to detect polymorphisms (different forms) of the DNA (Williams *et al.*, 1990). Polymorphism is due to the matching of the primer sequence to a complementary sequence on the target DNA; without a match, no binding will occur, and hence, there will be no amplification of the DNA. Polymorphisms are detected by the presence, or absence, of DNA products observed in a gel after electrophoresis (Williams *et al.*, 1990). RAPD analysis has been applied widely to assess genetic variation in trees, including *Gliricidia* (Chalmers *et al.*, 1992), *Populus* (Liu and Furnier, 1993) and *Eucalyptus globulus* (Nesbitt *et al.*, 1995).

PCR is particularly suitable for genetic studies of tropical tree species because only very small quantities (mg) of low quality leaf material can supply the DNA required. Apart from facilitating collection in the field, this also allows the analysis of leaf fragments from previously collected herbarium specimens.

7.3   Applications of molecular analyses in *Calliandra*

Molecular approaches have been used to address a number of important biological questions at different levels in *Calliandra*. The analyses can be grouped into three main areas: (1) the assessment of inter-specific hybridisation between *C. calothyrsus* and *C.

houstoniana; (2) ecogeographical variation within *C. calothyrsus*; and (3) reproductive biology. Each of these areas is considered separately, and the results discussed in terms of the implications for improved genetic resource management within *Calliandra* series *Racemosae*.

Inter-specific hybridisation

Hybridisation is associated with both taxonomic confusion and the potential loss of valuable species. A survey of sympatric populations of *C. calothyrsus* and *C. houstoniana* revealed that putative inter-specific hybrids were found rarely, despite an overlap in the individual species' flowering times (Chamberlain, 1996c). The two species were found generally to occur in separate 'micro-environments' that could favour one species over another and would select against any hybrids. In Flores, Guatemala, however, trees of intermediate morphology were found in areas containing *C. houstoniana* subsp. *houstoniana*, *C. calothyrsus* and *C. houstoniana* subsp. *stylesii* where all three species were intimately mixed. It was hypothesised that habitat disturbance following human activity, e.g., agriculture and road construction, in combination with the pioneer nature of these species, may have led to hybridisation between previously separated taxa.

In order to identify the taxonomic status of the putative hybrid form at Flores, leaf samples from herbarium specimens and plant material collected at Flores of *C. calothyrsus*, *C. houstoniana* and the intermediate form were selected for molecular analysis using RAPD markers (10 samples per taxon; Hubert, 1997). The aim of the study was to identify species-specific RAPD markers that could be compared with markers from the intermediate form. Additivity of the parental markers in the intermediate taxon would suggest a hybrid origin (Hawkins and Harris, 1998; Hughes and Harris, 1998).

Table 7.1:   Diagnostic RAPD markers for *Calliandra calothyrsus* and *Calliandra houstoniana* subsp. *houstoniana* from Flores, Guatemala, expressed as frequencies (1.00 = fixed, 0.00 = absent).

Primer	RAPD product	*C. calothyrsus*	*C. houstoniana*	Intermediate
OP-A05	790	1.00	0.00	0.00
OP-A11	860	1.00	0.00	0.29
OP-A16	870	0.00	1.00	0.89
OP-A17	650	0.00	1.00	0.33
OP-B05	710	0.00	1.00	0.11
	910	1.00	0.00	0.00
OP-B11	900	1.00	0.00	0.57

The species of *Calliandra* series *Racemosae* analysed with RAPD markers were revealed to be highly polymorphic (Hubert, 1997). Diagnostic markers[2] were found that distinguished *C. calothyrsus* at Flores from *C. houstoniana* subsp. *houstoniana* (Table 7.1). However, these markers were not always additive in the intermediate form. This has been observed for other hybrid plant species (see Hawkins and Harris, 1998; Smith *et al.*, 1996; Wang *et al.*, 1994) and may be due to sampling effects, or uniparental inheritance if the marker is of chloroplast or mitochondrial origin.

Principal component analysis revealed the RAPD marker similarities between *C. calothyrsus*, *C. houstoniana* and the intermediate form. Figure 7.1 shows two groups, one containing *C. houstoniana* and the intermediate forms, and the second containing the majority of the *C. calothyrsus* samples. The intermediate form appears to be more similar to *C. houstoniana*, however, this is in contrast to a morphological analysis of the putative hybrid material from Flores (Hubert, 1997) which suggested that the intermediate form was more similar to *C. calothyrsus* (see Chapter 3). This apparent contradiction has also been noted in an investigation of *Eucalyptus risdonii* and *E. amygdalina* hybrids occurring in Tasmania (Sale *et al.*, 1996). There could be a number of reasons for this, but one possibility is that morphology may not necessarily reflect the genetic contribution of the two parental species (Rieseberg and Ellstrand, 1993). If phenotypic differences were determined by relatively few genes then these differences may not be reflected in the RAPD data.

The use of both morphological and molecular techniques for the identification of natural inter-specific hybrids within *Calliandra* series *Racemosae*

was effective (Hubert, 1997). Both approaches suggested a hybrid origin of the intermediate form found at Flores, Guatemala, although the means by which the hybrid may have arisen and its parentage were not conclusively determined.

The identification of this hybrid has important implications for the genetic integrity of seed collected from Flores, or from other sites of sympatry. Ideally, seed should be collected from stands of *C. calothyrsus* that are isolated from other *Calliandra* species to avoid contamination with undesirable pollen, and prevent the sampling of hybrids. In particular, seed should not be collected from stands where trees from more than one species are intimately mixed within a few metres of one another. Similarly, *ex situ* conservation stands or seed production orchards should be isolated from other species of *Calliandra*, although since *C. calothyrsus* is the only species widely used as an exotic, this should not be a major concern.

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[2] Taxon-diagnostic markers are defined as invariant and fixed in one taxon, and absent from another. If RAPD makers are dominant and inherited in a Mendelian fashion, then products that are fixed for one species should appear in the F1 hybrid.
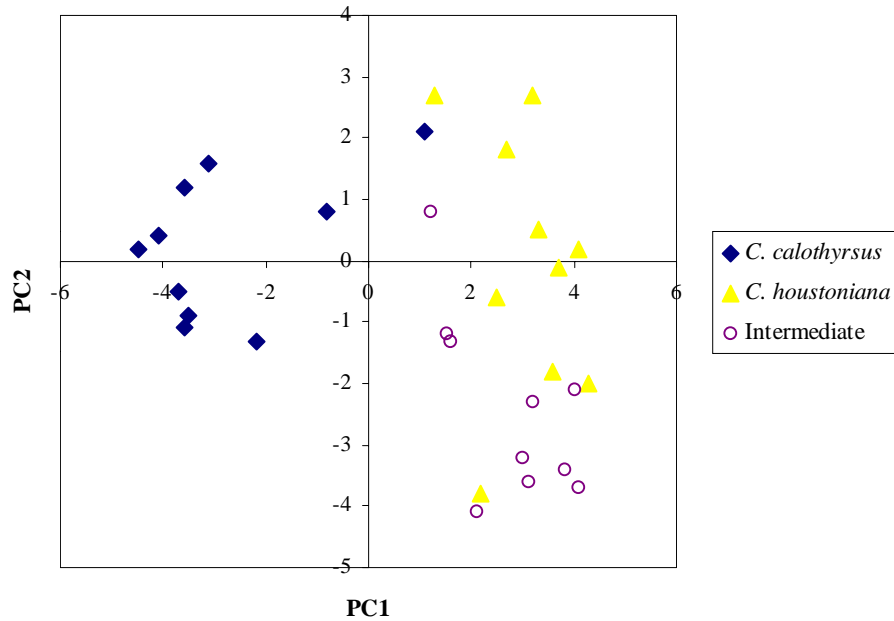
Figure 7.1:   PCA plot of the first two principal components for *Calliandra calothyrsus*, *C. houstoniana* and a putative hybrid (Intermediate).

Ecogeographical variation within *C. calothyrsus*

Fundamental to the efficient utilisation and conservation of *C. calothyrsus* is an understanding of the relative distribution of genetic variation within and between populations, or regions, across a wide geographical range. In addition to this information, knowledge of the absolute levels of variation within populations is crucial for outcrossing tree species such as *C. calothyrsus*, since this has implications for the sustainability of resource utilisation after release to farmers. Diversity and field performance may be linked in outcrossing trees (Griffin and Lindgren, 1985; Mitton and Jeffers, 1989; Park and Fowler, 1982), and the release of genetically diverse *C. calothyrsus* may therefore be necessary to prevent inbreeding depression during future generations. In addition, high diversity is important because it may provide the capacity to adapt to varying farmer requirements and environmental conditions (Simons *et al.*, 1993).

Isozyme analysis within and between populations

In order to assess the distribution of genetic variation within and between populations of *C. calothyrsus* over a wide eco-geographical range, Chamberlain (1998a; 1996a) employed isozyme analysis on fifteen populations representative of the species' natural distribution in Mesoamerica, and seven exotic populations from Australia, Indonesia, Jamaica and Kenya (Table 7.2). Ten enzyme systems were used to resolve isozymes and 23 loci, polymorphic either within or between populations, were employed to assess

diversity. Estimates of diversity (heterozygosity, H^3) within populations, calculated according to Nei (1978), are shown in Table 7.2.

Natural populations: A high heterozygosity value implies a high level of genetic diversity. Of the populations sampled, Zihuatanejo, Mexico was the most diverse, with those from Barillas, Bombana, Fortuna and El Volcán exhibiting the lowest heterozygosity values. Levels of isozyme variation found within natural populations of *C. calothyrsus* (mean $H_o = 0.057$) were relatively low when compared to other animal-pollinated, early-successional and widespread tropical tree species (Hamrick and Godt, 1989; Loveless, 1992; Loveless and Hamrick, 1984). This may be due to selection pressures experienced by populations in their natural habitats, or to human disturbance. In areas of secondary vegetation, *C. calothyrsus* often occurs in small, scattered stands, which may be frequently cut or burnt by local people. This could result in the loss of alleles through genetic drift. For example, in 1995, the population at Copán, Honduras was almost entirely destroyed due to the upgrading and widening of a nearby road, leaving only a few scattered individuals.

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[3] H_o is the proportion of individuals observed to be heterozygous, averaged across all loci. H_e is the proportion of heterozygous individuals expected if the population were in Hardy-Weinberg equilibrium. Large differences between H_o with H_e can indicate non-random mating in a population.

Table 7.2:   Collection site data and mean heterozygosities (*H*) averaged over 23 isozyme loci for 22 populations of *Calliandra calothyrsus* sampled from a wide ecogeographical range in Mesoamerica and from exotic introductions (number of individuals per family or bulk seedlot and standard errors for heterozygosities are in parentheses; Chamberlain, 1998a).

Population	Lat (N)	Long (W)	No. families	Mean heterozygosities (*H*)	
				Observed (H_o)	Expected (H_e)
Native populations:					
Bombana, Mexico	16°56'	93°02'	20 (5)	0.008 (0.006)	0.013 (0.008)
Zihuatanejo, Mexico	17°40'	101°30'	bulk (60)	0.115 (0.041)	0.119 (0.037)
Barillas, Guatemala	15°46'	91°19'	14 (5)	0.001 (0.001)	0.001 (0.001)
Cobán, Guatemala	15°28'	90°15'	20 (5)	0.046 (0.022)	0.076 (0.028)
Santa María de Jesus, Guatemala	14°45'	91°32'	15 (5)	0.068 (0.035)	0.073 (0.037)
Patulul, Guatemala	14°24'	91°09'	16 (5)	0.051 (0.022)	0.080 (0.033)
La Ceiba, Honduras	15°43'	86°50'	18 (6)	0.064 (0.030)	0.078 (0.035)
Copán, Honduras	14°50'	89°08'	bulk (60)	0.066 (0.028)	0.090 (0.038)
Meambar, Honduras	14°58'	87°46'	bulk (60)	0.095 (0.035)	0.112 (0.042)
Santa María, Honduras	14°07'	86°12'	bulk (60)	0.105 (0.036)	0.117 (0.039)
San Ramón, Nicaragua	12°54'	85°48'	13 (6)	0.077 (0.032)	0.089 (0.037)
La Puerta, Nicaragua	12°11'	85°15'	11 (7)	0.077 (0.031)	0.096 (0.039)
Fortuna, Costa Rica	10°30'	84°48'	12 (7)	0.009 (0.007)	0.014 (0.012)
Turrialba, Costa Rica	9°55'	83°42'	25 (5)	0.087 (0.046)	0.117 (0.050)
El Volcán, Panama	8°45'	82°24'	bulk (60)	0.008 (0.006)	0.010 (0.007)
Exotic populations:					
Maduin, Indonesia	7°36'S	111°30'E	bulk (60)	0.051 (0.024)	0.098 (0.040)
Sumba, Indonesia	9°50'S	120°05'E	bulk (60)	0.043 (0.018)	0.089 (0.030)
CSIRO, Australia	19°25'S	146°75'E	bulk (60)	0.043 (0.021)	0.090 (0.036)
Buff Bay, Jamaica	18°15'	76°04'	bulk (60)	0.100 (0.039)	0.117 (0.041)
Cinchona, Jamaica	18°06'	76°39'	bulk (60)	0.039 (0.039)	0.047 (0.030)
Hall's Delight, Jamaica	18°05'	77°04'	bulk (60)	0.024 (0.014)	0.029 (0.016)
Embu, Kenya	0°30'S	37°21'E	bulk (60)	0.047 (0.020)	0.099 (0.040)

Partitioning of genetic variation within and between populations according to Wright's *F*-statistic (Wright, 1951) indicated that most variation (~80%) occurred between rather than within the 22 populations sampled. A dendrogram based on Nei's (1978) unbiased genetic distances was used to visualise the partitioning of genetic variation in *C. calothyrsus* (Figure 7.2; Chamberlain, 1998a).

The dendrogram defines four main clusters:

♦ *C. calothyrsus-1* - 13 populations from Mexico, Guatemala, Honduras and exotic locations.

♦ *C. calothyrsus-2* - three populations from Costa Rica and Panama.

♦ *C. calothyrsus-3* - five populations from the inland, upland areas of Honduras and Nicaragua.

♦ *C. calothyrsus-4* - the population from Zihuatanejo, Mexico.

The definition of these clusters was also supported by population aggregation analysis (Chamberlain, 1998a; Davis and Nixon, 1992), and provided evidence for the existence of distinct subgroups within the species. These population clusters correspond closely to broad geographical, ecological, and morphological boundaries (Chamberlain *et al*., unpublished results) and are described in detail in Chapter 2.
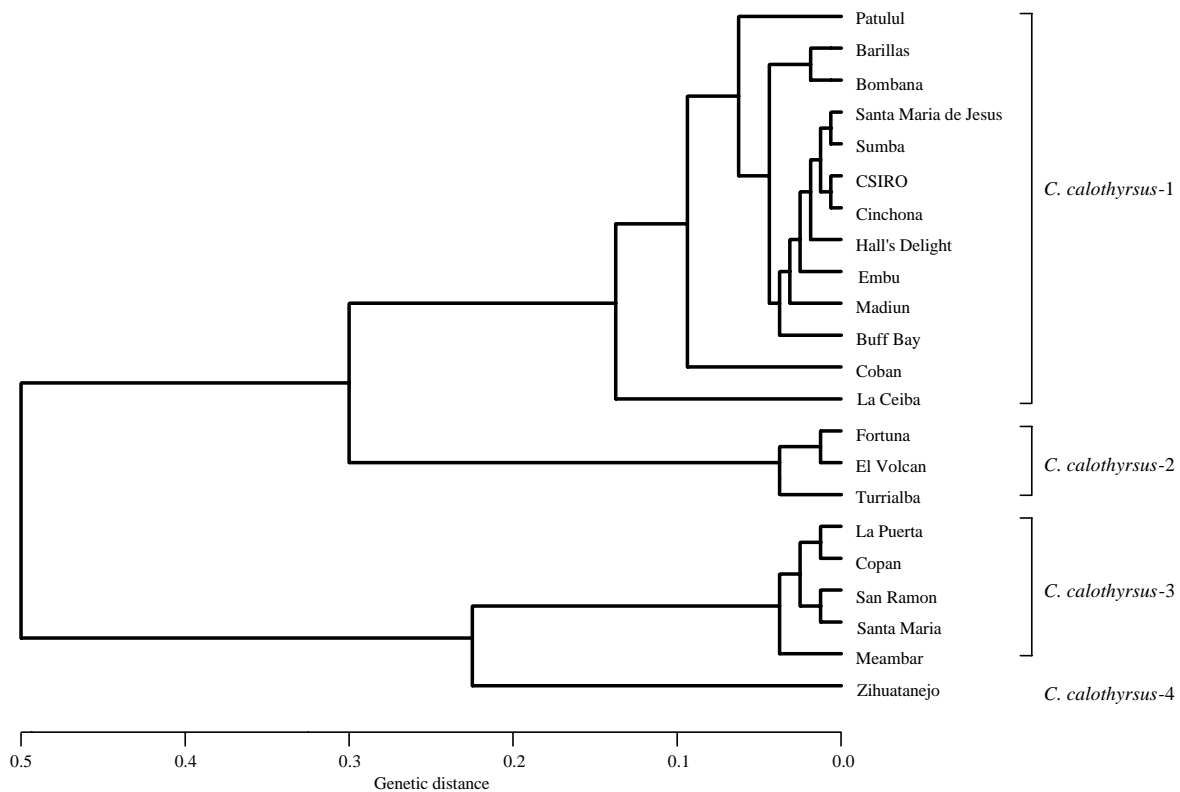
Figure 7.2: Dendrogram showing isozyme similarities between 22 populations of *Calliandra calothyrsus*.

Exotic populations: C. calothyrsus is known to have been introduced to Indonesia from Guatemala by Dutch botanists in 1936 (Verhoef, 1939). It was introduced as coffee shade, along with a number of other legume species, as a substitute for *Leucaena leucocephala*. Isozyme variation has shown that the exotic populations of *C. calothyrsus* are most similar to the populations from Mexico and Guatemala, particularly the population from Santa María de Jesus, Guatemala. It is therefore likely that seed from this region of Guatemala was introduced to Indonesia and from there to other exotic locations.

Levels of isozyme variation found within exotic populations of *C. calothyrsus* (mean $H_o = 0.050$) were also low, but the populations from Australia, Indonesia and Kenya exhibited heterozygosities that were significantly lower than expected by the Hardy-Weinberg equilibrium, i.e., possessed H_e values that were significantly different from the corresponding H_o value. The disequilibrium observed may have resulted from inadequate sampling of the populations for seed, or non-random mating through selfing or mating between related individuals. Selfing has been shown to occur at a substantial frequency in some populations of *C. calothyrsus* (see Chapter 3), which violates one of the major assumptions of the Hardy-Weinberg principle. In such a case, the frequency of homozygous genotypes in the population will increase at the expense of heterozygous genotypes, and heterozygosity will be lower than expected. In contrast, the heterozygosities of the three exotic populations from Jamaica suggested random mating was occurring and diversity is being maintained. The population from Buff Bay, Jamaica was the most diverse exotic population, and was more diverse than many of the native populations from Mexico and Central America.

The analysis of isozyme variation in *C. calothyrsus* has revealed a large amount of genetic variation, particularly between populations. Extensive eco-geographical sampling is therefore important for the optimal capture of diversity and the conservation and utilisation of the species.

Mating system and gene flow

Information on the mating system of *C. calothyrsus*, and the patterns of gene flow within its native populations, can influence population genetic structure and the way in which populations will develop following the introduction of wild material into exotic locations (Loveless and Hamrick, 1984; Schaal, 1980).

Mating system of C. calothyrsus.

To assess the degree of outcrossing in *C. calothyrsus*, Chamberlain (1997) collected seed from 44 individual trees in five sub-populations within the River Bonito, Colón, Honduras. Isozyme electrophoresis was employed to examine 20 progeny from each tree in the population. The mixed mating model of Brown *et al.* (1985) was used, employing a maximum likelihood approach (Ritland and Jain, 1981) and the MLT computer program (Ritland, 1990), to estimate the outcrossing rate at this site.

The outcrossing rate may theoretically range from zero (completely selfed) to one (completely outcrossed), although values greater than one may occur if there is preferential mating between unrelated genotypes, or if there are a large number of heterozygous maternal genotypes. Two population outcrossing rates were estimated: t_m, the multilocus outcrossing rate, in which data from all loci are analysed together; and t_s, the single locus outcrossing rate, where each locus is analysed separately. Inferences about inbreeding other than selfing, i.e., mating between related individuals, can be made from comparisons between these two outcrossing rate estimates. Outcrossing rates for the sub-populations and the population as a whole are shown in Table 7.3. Both the mean t_s over all loci (estimated as 0.634 (SE 0.092)) and t_m (estimated as 0.687 (SE 0.075)) differed significantly from 1. This

suggested that *C. calothyrsus* at this site possesses a mixed mating system, and the lack of a significant difference between t_m and t_s suggested that the inbreeding observed was due to selfing. This result concords with analyses of the breeding system which suggested *C. calothyrsus* can tolerate selfing and has a relatively weak self-incompatibility mechanism (Chamberlain, 1998b; see Chapter 3).

Mixed mating systems in plants are considered to be evolutionarily stable and may confer both the advantages of selfing (avoidance of recessive deleterious alleles) and outcrossing (avoidance of inbreeding depression) to a population (Holsinger, 1991). *C. calothyrsus* seed selected for the establishment of seed production orchards should, however, be collected from widely-spaced mother trees in the original population to reduce the likelihood that they are related. Outcrossing will be promoted in seed orchards and diversity maintained in the seed crop. In natural populations, the potential for inbreeding in *C. calothyrsus* may be high as a result of severe disturbance through human activity. The species is widespread and thrives in disturbed habitats, but excessive disturbance, e.g., as experienced by the population at Copán, Honduras (see above), may lead to inbreeding depression and the loss of important resource populations.

Table 7.3:   Maximum likelihood estimates of the single locus (t_s) and multilocus (t_m) outcrossing rates using ten polymorphic isozyme loci for five sub-populations of *Calliandra calothyrsus* in Honduras (standard errors in parentheses; Chamberlain, 1997).

Sub-population	No. of families	F	t_m	t_s	t_m - t_s
1	14	-0.303 (0.292)	0.635 (0.140)	0.655 (0.133)	-0.020 (0.014)
2	4	-0.019 (0.301)	1.155 (0.301)	1.655 (0.507)	-0.500 (0.422)
3	6	0.335 (0.281)	0.136 (0.142)	0.095 (0.139)	0.041 (0.016)
4	5	-0.149 (0.306)	0.176 (0.124)	0.116 (0.103)	0.060 (0.033)
5	15	0.260 (0.349)	0.798 (0.108)	0.692 (0.173)	0.106 (0.073)
All	**44**	**-0.116 (0.354)**	**0.687 (0.075)**	**0.634 (0.092)**	**0.053 (0.040)**

Pollen-mediated gene flow

Pollen-mediated gene flow is recognised to play an important role in determining the genetic structure of populations and has important *ex situ* implications for *C. calothyrsus* within conservation stands and seed orchards. Its estimation by observations of pollinator movement are difficult because of the phenomenon of 'carry-over', by which pollen may be transferred across several plants before final deposition on the stigma (Levin, 1978; Schaal, 1980), and the logistics of pollinator trapping through capture, mark and recapture. Therefore, in order to provide a reliable estimate of pollen-mediated gene flow, Chamberlain (unpublished data) employed isozyme analysis to assess the seed collected from the Honduran population described above. Since every parental tree in this population was characterised for ten isozyme loci, and since the geographical position of every tree was also recorded, comparison of progeny arrays with parental data allowed calculation of pollen flow distances by paternity exclusion analysis (Devlin and Ellstrand, 1990).

For the whole population, subsets of possible fathers (pollen donors) were derived as a function of increasing distance from the mother tree (< 50 m, <

100 m, … , < 2400 m). The analysis generated a leptokurtic distribution curve that suggested the majority of pollen received by an individual tree came from a tree within a 200 m radius of the mother tree (Figure 7.3). More rarely, pollen was received from up to 2400 m from the mother tree. A rare allele (*Pgm*-1c) that only occurred in two of the sub-populations separated by an approximate distance of 2500 m also suggested that there was pollen flow over distances greater than 2 km. Paternity exclusion analysis was also performed for each individual sub-population. The pollen flow distances in each sub-population were also approximately leptokurtic, with most of the pollen again originating from within 200 m of the mother tree, i.e., from within the sub-population. However, a significant proportion of the pollen received by each sub-population originated from trees in the nearest sub-population. For example, in sub-population three, 72% of pollen was likely to have originated from trees within a 200 m radius of the mother tree, but 15% of pollen was likely to have come from trees 500-700 m away, i.e., in sub-population two. This suggests that population structure can have a significant effect on pollen flow, and the potential for long-distance pollen flow, although rarer, may have a significant effect on seed paternity.
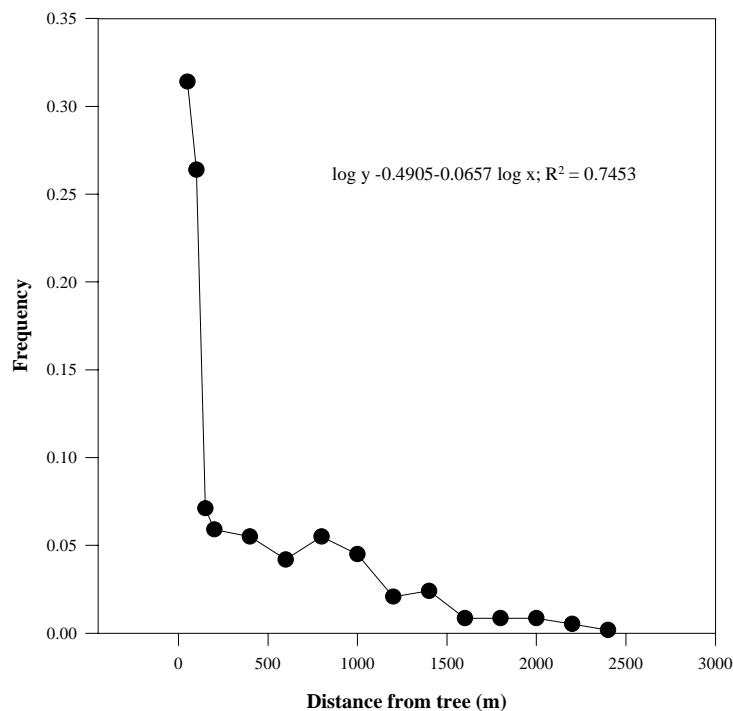

log y -0.4905-0.0657 log x; $R^2 = 0.7453$

Figure 7.3:   Pollen flow distances in *Calliandra calothyrsus* in the River Bonito, Colón, Honduras.

Key findings from molecular analyses and their implications

Hybridisation between *C. calothyrsus* and *C. houstoniana*.

♦   RAPD-based analyses indicated inter-specific hybridisation at Flores, Guatemala, where three species, *C. calothyrsus*, *C. houstoniana* subsp. *houstoniana* and *C. houstoniana* subsp. *stylesii*, are intimately mixed.

Implications: There is the potential for the loss of genetic integrity in important native populations of *C. calothyrsus* through hybridisation. Care is required when sampling such populations for seed, and *ex situ* conservation stands and seed production orchards should be isolated from other species of *Calliandra*.

Genetic variation within and between natural populations of *C. calothyrsus*.

♦   Levels of isozyme variation found within populations of *C. calothyrsus* (mean H_o = 0.057) were relatively low when compared to other animal-pollinated, early-successional and widespread tropical tree species

♦   The level of isozyme variation found between populations of *C. calothyrsus* (mean F_{ST} = 0.802) was high and indicated four distinct sub-groups with different regional distributions and morphology.

Implications: Widespread sampling of unrelated individuals within populations, as well as extensive collection throughout the native range, is critical for the optimal capture of genetic variation for both conservation and utilisation.

Genetic variation within and between exotic populations of *C. calothyrsus*.

♦   Levels of isozyme variation found within the exotic populations of *C. calothyrsus* (mean H_o = 0.050) were relatively high when compared to the native populations, although there are departures from Hardy-Weinberg equilibrium.

♦   Isozyme analysis showed that the exotic populations of *C. calothyrsus* were most similar to the populations from Mexico and Guatemala, particularly the population from Santa María de Jesus.

Implications: *C. calothyrsus*, cultivated in exotic locations, originated from the Pacific slopes of Guatemala in the vicinity of the population at Santa María de Jesus. This material generally possesses moderate levels of variation and has the capacity to adapt to varying environments.

Mating system and gene flow.

♦   Isozyme analysis of individuals within a population from Honduras suggests that *C. calothyrsus* possesses a mixed mating system.

♦   Paternity analysis suggested that the majority of pollen is dispersed over distances of less than 200 m, but occasionally more than 2500 m, allowing gene flow over this distance. Pollen flow will be influenced by population structure.

Implications: It is important to minimise selfing and related matings in *C. calothyrsus* to avoid inbreeding depression. For the establishment of *ex-situ* conservation stands or seed production orchards, seed must be collected from widely spaced, unrelated trees. Stands of superior genetic material should be separated from existing populations by at least 2 km.

The results from the molecular analyses of genetic variation have described a wealth of diversity within *C. calothyrsus* and its close relatives. The results also have important implications for the species' management. Ideally, seed should be collected from stands of *C. calothyrsus* that are isolated from other *Calliandra* species to prevent the sampling of hybrids. Similarly, *ex situ* conservation stands or seed production orchards should be isolated from other species of *Calliandra*, or inferior land race material.

Species and provenance integrity is normally ensured by an isolation zone that separates orchard trees from neighbouring stands of related or the same species (Xie and Knowles, 1994). An analysis of *C. calothyrsus* pollinator behaviour suggested that isolation distances should be not less than 2000 m (Chamberlain and Rajaselvam, 1996; see Chapter 3). The maximum pollen transfer distance of 2400 m observed using isozyme markers concords with the observations made by Chamberlain and Rajaselvam and suggests that isolation zones should be at least this wide if integrity is to be maintained in a seed orchard. In addition, the pollen flow data has implications for the introduction of superior provenances into exotic locations where local sub-optimal land races already exist as a result of previous introductions, because trees less than 200 m apart could readily exchange pollen. Although the full implications of such interactions in *C. calothyrsus* are not presently well understood, one result could be a dilution of the favourable gene complexes in subsequent generations of a superior provenance, leading to a gradual loss of advantage over the pre-existing material.

8   Conservation

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8.1   Introduction

A goal of conservation is to maintain biological diversity in the long-term. Conservation efforts can be focused at a number of different levels, for example, the protection of multiple ecosystems, single ecosystems, genera, species, or their varieties. The conservation of plant species, perhaps rare or endangered, may only be ensured through the conservation of their habitats, i.e., by establishing protected reserves. For widespread or abundant species, attention needs to focus on the levels of biological diversity within them (Millar and Libby, 1991).

During the early years of plant genetic conservation, the species level of diversity received the most research attention. Since then, conservation biology has advanced rapidly in its understanding of species and population biology, and it has been recognised that the unique, rare and unusual variation that exists at intra-specific levels in most species cannot be ignored (Graudal *et al.*, 1997; Maxted *et al.*, 1997; Vogler and Desalle, 1994).

Calliandra calothyrsus is not an endangered species within its native range being widespread across a diverse range of habitats, and sometimes locally abundant. The species often thrives on recurrent disturbance and can be a common element of secondary vegetation. However, some important resource populations, often composed of small numbers of individuals, are vulnerable to erosion and potential loss. These populations may have been exposed to genetic bottlenecks, both now and in the past, as a result of extreme disturbance. The recovery and genetic status of these important resource populations may be difficult to predict.

The purpose of this chapter is to illustrate how the genetic diversity of *C. calothyrsus* can be used to develop and prioritise conservation efforts for the species.

8.2.   Conservation of *C. calothyrsus*

In the case of extremely rare plants, limited to a few small populations, the goal of conserving the entire species may be possible through habitat protection and *ex situ* preservation. For widespread species, such as *C. calothyrsus*, conservation will involve protecting appropriate samples of the whole species. The goal in this case is to choose samples such that the important and interesting genetic variation in the species is conserved. Such sampling decisions can be based on information obtained from studies on the genetic structure of populations.

Genetic structure of *C. calothyrsus*

The genetic structure of *C. calothyrsus* has been described by the use of a suite of molecular, morphological, and agronomic characters. These patterns of variation have been described in detail elsewhere (Chamberlain 1996a; 1998a; Chamberlain *et al.*, unpublished results; see Chapters 2 and 7), but are summarised here for the purpose of prioritising populations of the species for conservation.

The analysis of isozyme and RAPD molecular markers has provided evidence for the existence of high levels of between-population variation within *C. calothyrsus* (high F_{ST} values), accompanied by low levels of within-population variation (low H_o values; Table 8.1; Chamberlain, 1998a; Hubert, 1997). This led to the description of four sub-groups within *C. calothyrsus* (*C. calothyrsus-1* to *-4*) based on distinct morphological variation that paralleled the observed variation in molecular markers. Differences in environment (geographical location, altitude, rainfall) were in turn associated with particular combinations of molecular and morphological variation, and further supported the description of four groups within the species (Chamberlain *et al.*, unpublished results).

Further genetic variation was observed within the sub-groups of *C. calothyrsus* (Table 8.1; Chamberlain, 1998a). *C. calothyrsus-1* and *-2* were typified by relatively high between-population and low within-population variation (mean $H_o = 0.038$; mean $F_{ST} = 0.397$), as for the species as a whole, whereas *C. calothyrsus-3* and *-4* were typified by higher levels of within-population variation (mean $H_o = 0.099$), and low between-population variation in the case of *C. calothyrsus-3* ($F_{ST} = 0.086$).

Table 8.1:   A summary of genetic diversity estimates for 15 native populations of *Calliandra calothyrsus* surveyed with isozyme markers (Chamberlain, 1998a: N – number of populations, P - percentage polymorphic loci, A - average number of alleles, H_o - observed heterozygosity and F_{ST} - Wright F-statistic; standard errors in parentheses).

Sub-group	N	P	A	H_o	F_{ST}
C. calothyrsus-1	6	18.48	1.28 (0.11)	0.042 (0.020)	0.469
C. calothyrsus-2	3	13.05	1.15 (0.07)	0.035 (0.020)	0.327
C. calothyrsus-3	5	25.22	1.41 (0.13)	0.084 (0.032)	0.086
C. calothyrsus-4	1	34.78	1.61 (0.15)	0.115 (0.041)	-
Mean	**15**	**20.46**	**1.22 (0.10)**	**0.057 (0.024)**	**0.802**

Abrupt genetic differences between populations (discontinous variation) may occur as a result of natural selection and/or genetic drift. When selection appears to be the primary factor leading to differentiation, as suggested by the association between major differences in environment and particular combinations of genetic variation, ecotypes can be recognised (Read 1980). In *C. calothyrsus*, an essentially ecotypic pattern of variation can be observed with patterns of variation in terms of molecular and morphological characters being associated with particular geographical locations and their ecology. The variation observed may be maintained by the isolation of disjunct populations through little or no gene flow between them and subsequent genetic drift.

The discontinous variation observed in *C. calothyrsus* therefore suggests that a conservation strategy for the species should aim to not preserve the entire species *per se*, but to conserve genetically diverse populations from the hierarchy of sub-groups and their populations described above (Millar and Libby, 1991). Because the existence of *C. calothyrsus* is not threatened, the aim should be to safeguard the evolutionary fitness of the species, and avoid genetic erosion within it (Templeton, 1991).

Priorities for the conservation of *C. calothyrsus*

The highest priority, especially within *in situ* conservation, is to capture the core of variability, i.e., populations that contain the common or shared genetic variation for a particular region. Hence, the genetic structure of *C. calothyrsus*, i.e. the high between- and low within-population variation can be used to prioritise populations for conservation. As *C. calothyrsus* is an economically important tree species,

populations containing the most valuable trees, i.e., those that are most productive in terms of growth and biomass accumulation, could also be considered to be of high priority for conservation.

The most widely accepted measure of conservation status, or threat, comes from the IUCN Red List Categories (IUCN, 1994). These are widely known and have been in use for over 30 years (IUCN, 1994; Wang, 1996). A category of threat is assigned to a taxon based on any one of five criteria relating to taxon range, population size and rate of decline. The system can be applied to any taxonomic unit at or below the species level, and to any geographical or political area. The IUCN system has been most widely adopted at the species level, however, and rarely at the population level (Avery *et al.*, 1995; Hughes, 1998; Kirchhofer, 1997; Poulsen and Krabbe, 1997). For *C. calothyrsus*, IUCN ratings were assigned to the species as a whole, and to the four subgroups within it (Table 8.2). *C. calothyrsus-4* was given the highest priority for conservation because of its narrow distribution, and populations composed of fragmented and scattered remnants of often only a few individuals.

Another method of rating species or taxa for conservation concern is provided by Hawthorne (1996), and has been utilised to define the conservation status of *Leucaena* species (Hughes, 1998). This system is called the Star Rating method and adopts a slightly different approach to the IUCN system. Star ratings are defined and assigned initially on the species global distribution and modified subsequently by the species' regional and local abundance, ecology, taxonomy, life history traits and interactions with the ecosystem. The Star Rating system takes into account more distribution factors and species characteristics than does the IUCN system. Using this system, *C. calothyrsus-4* was again most highly prioritised for

conservation, but *C. calothyrsus-2* with its restricted distribution in Costa Rica and Panama, received a higher prioritisation than *C. calothyrsus-1* or *C. calothyrsus-3* (Table 8.2).

Graudal *et al.* (1997) described a comprehensive strategy for planning conservation programmes for forest genetic resources. Their approach is orientated to species of socio-economic value and allows populations of these species to be identified for conservation. As with the Star Rating system, a great deal of information relating to species characteristics is taken into account when prioritising populations for conservation, including the species' distribution,

ecology, life history traits, occurrence of populations in protected areas, the effects of selective exploitation or disturbance, and the location of populations known to be valuable. Emphasis is also placed on the need to conserve intra-population diversity through conserving an appropriate number of individuals per population, which takes into account sub-population structure and environmental factors.

The strategy employed by Graudal *et al.* (1997) was also used to identify specific populations of *C. calothyrsus* for conservation (Table 8.3).

Table 8.2:   Conservation status of *Calliandra calothyrsus* and its subgroups.

Species/subgroup	IUCN Threat category[1]	Star Rating[2]	Notes
C. calothyrsus	LR: lc	Green	Widespread and often locally abundant; present in several protected areas, but high levels of infra-specific variation in the species warrants conservation below the species level.
C. calothyrsus-1	LR: lc	Green	Widespread and often locally abundant in Mexico, Guatemala, and Honduras; present in several protected areas; sometimes cultivated as coffee shade.
C. calothyrsus-2	LR: lc	Blue	Widespread in Costa Rica and Panama and often locally abundant; present in several protected areas.
C. calothyrsus-3	LR: lc	Green	Widespread, occasionally locally common in bush fallow in Guatemala, Honduras and Nicaragua; susceptible to genetic erosion through human disturbance.
C. calothyrsus-4	VU: C2a	Gold	Restricted to 200 km^2; populations composed of fragmented and scattered remnants of few individuals; often many isolated individuals occur and poor seed set has been reported (Macqueen, 1993).

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[1] IUCN Red List Categories based on IUCN (1994): VU: C2a = Vulnerable, population less than 10,000 individuals and severely fragmented; LR : lc = Lower Risk, least concern.
[2] Star Rating based on Hawthorne (1996): Gold = moderate conservation concern; Blue = minor conservation concern; Green = no conservation concern.

Table 8.3: Twelve populations of *Calliandra calothyrsus* prioritised for conservation based on their potential productivity and value for planting within farming systems, and which are representative of the variation in the species as a whole (after Graudal *et al.*, 1997).

Subgroup	Population	Notes
C. calothyrsus-1	Georgesville, Belize	Moderately-sized population at forest reserve boundary; good prospects for *in situ* conservation.
	Gracie Rock, Belize	Small population colonising a roadside and adjacent cattle pasture over 1-2 km; potential for *circa situm* conservation in bush fallow.
	Barillas, Guatemala	Extensive population scattered in roadsides and secondary vegetation; potential for *circa situm* conservation in bush fallow; conserved *ex situ* in long-term seed storage.
	Flores, Guatemala	Extensive population scattered in roadsides and cattle pasture on the flat plains of Lake Petén Itza; at threat from genetic erosion due to human disturbance through road building; conserved *ex situ* in long-term seed storage.
	Patulul, Guatemala	Small population that has seen a reduction in the number of its mature individuals over the last five years; *circa situm* conservation as coffee shade and bush fallow; conserved *ex situ* in seed orchards and long-term seed storage.
	Santa María de Jesus, Guatemala	Small population scattered in roadsides, river margins and restricted use as coffee shade; *circa situm* conservation as coffee shade; conserved *ex situ* in seed orchards.
	Plan del Rio, Mexico	Moderately large population found in disturbed riparian forest and adjacent agricultural fields; potential for *circa situm* conservation in bush fallow.
	Union Juarez, Mexico	Small population found over coffee, in roadsides or stream beds; potential for *circa situm* conservation as coffee shade.
C. calothyrsus-2	Fortuna, Costa Rica	Fragmented population with individuals found in three main sub-populations over a distance of 100 km^2; largest sub-population secure in riparian forest; conserved *ex situ* in seed orchards.
	Turrialba, Costa Rica	Moderately-sized in disturbed riparian forest; good prospects for *in situ* conservation.
C. calothyrsus-3	La Puerta, Nicaragua	Moderately large population colonising roadsides and adjacent cattle pasture; could be susceptible to genetic erosion; potential for *circa situm* conservation in bush fallow.
	San Ramón, Nicaragua	Small population colonising roadsides and adjacent cattle pasture; *circa situm* conservation through propagation for seed and bush fallow; conserved *ex situ* in seed orchards and long-term seed storage.
C. calothyrsus-4	Zihuatanejo, Mexico	Small population composed of fragmented and scattered remnants of few individuals; potential for *circa situm* conservation in bush fallow; should be conserved *ex situ*.

Conservation measures

The species samples chosen for conservation can be protected *in situ* and/or by *ex situ* germplasm preservation (Frankel and Hawkes, 1975), although an integrated approach that involves both methods is generally preferred (Falk, 1987). There are obvious benefits to protecting genetically valuable populations through various levels of *in situ* management, but the *ex situ* component acts as a backup system to safeguard the variation in a native habitat, and may provide a source of germplasm for research, monitoring and restoration.

In situ conservation

Conservation within native habitats protects and perpetuates the integrity of gene pools and of co-adapted gene complexes. Individuals, populations and species interact with one another and with changing environments and thus maintain their evolutionary fitness. There are, however, many forms of *in situ* management with different conservation objectives having different priorities (Salwasser, 1987). Traditionally, *in situ* conservation has sought to maintain taxa in the 'natural state', e.g., in fully protected biological reserves or national parks. The impact on natural habitats, particularly forests, from agriculture and urban expansion means that for many species, *in situ* conservation in protected reserves is untenable. The adoption of *circa situm* conservation, i.e., the protection of species within native, managed environments, for example, tree species within farming systems, around settlements and in managed forests, is recognised as the only form of *in situ* conservation available for some species (Hughes, 1998; referred to as '*circa situm* conservation' by Kanowski and Boshier, 1997; 'locally based conservation' by Qualset *et al.*, 1997; 'farmer-based conservation' by Brush, 1991; or included with *in situ* conservation, e.g., by Bellon *et al.*, 1997; Graudal, *et al.*, 1997; Maxted *et al.*, 1997; Ortega, 1997). The economically important legume genus, *Leucaena*, is one such case being represented in its native range only within highly disturbed natural forest and farming systems (Hughes, 1998; Hughes *et al.*, 1995). Its *circa situm* conservation through use, i.e., the propagation and protection of trees on farms, is the only way many species of *Leucaena* and their populations can be conserved.

Biological reserves and national parks still remain an obvious form for the *in situ* preservation of species. For many widespread species, only a small percent of their populations, if any at all, will be included in such reserves. In the case of *C. calothyrsus*, only 10% of surveyed populations are found within biological reserves or national parks (Table 8.4). Often the species can be found thriving on the 'edges' of national parks, in areas that have been subjected to disturbance sometime in the past. However, only two of the distinct sub-groups within *C. calothyrsus* are represented in reserves, i.e., *C. calothyrsus-1* and *-2* with 15% and 16.5% of their respective populations. The majority of the populations of *C. calothyrsus-3* and *-4* are found colonising fallow agricultural fields, cattle pasture and roadside margins. These populations, especially those occurring predominantly in roadsides, are at greatest risk from extirpation.

For all the sub-groups of *C. calothyrsus*, therefore, but particularly for *C. calothyrsus-3* and *-4*, a strategy of *circa situm* conservation may be the appropriate option. The potential of this strategy for *C. calothyrsus* will be, however, dependent on the habitat type in which the species occurs. Table 8.4 illustrates the differing types of habitat in which *C. calothyrsus* is found, e.g., in river valleys and on lake margins (riparian habitats), on fallow agricultural land or cattle pasture, and on roadside margins. The majority of the riparian habitats are found within disturbed forest and are surrounded by cattle pasture or agricultural land, or run close to a road. Despite this, these areas can be considered to be relatively secure, as the forest is often maintained to protect the watershed and a shaded location is provided in which cattle can drink, or humans can utilise for recreational purposes.

In cattle pasture or fallow agricultural fields, *C. calothyrsus* often regenerates as a bush fallow. For example, the population at La Puerta, Nicaragua, is composed of thickets of *C. calothyrsus* regenerating in cattle pasture. Fields in this area are subjected to rotations of pasture grass and bush fallow, hence different fields will maintain the population of trees over different years, and the soils will be ameliorated by the N-fixing ability and leaf litter of *C. calothyrsus*. Conservation of *C. calothyrsus* may therefore be effected by this form of farming, but the impact of this type of management on the genetic structure and effective population size of the species is unknown. This system could be considered as a form of *circa situm* conservation, although the *C. calothyrsus* occurs as a derivative of the system rather than being nurtured and conserved for a particular purpose within the system.

Within the native range, *C. calothyrsus* occurs rarely in cultivation as shade over coffee. The species is not a popular shade tree in these areas, however, and generally, other legume genera, e.g., *Inga* and *Erythrina* are more common and preferred over *C. calothyrsus* (Arias and Macqueen, 1996).

Table 8.4:   Subgroups of *Calliandra calothyrsus* and habitat variation in the native range (after Macqueen, 1993).

	Percentage of populations in differing habitats			
Subgroup	Field margins and roadsides	Riparian habitats	Forest reserves	In cultivation
C. calothyrsus-1	25	45	15	15
C. calothyrsus-2	16.5	67	16.5	-
C. calothyrsus-3	55	45	-	-
C. calothyrsus-4	80	20	-	-
Total	**35.4**	**45.8**	**10.4**	**8.4**

Its conservation through use in this type of farming system can not, therefore, be assured in the long term. These populations do contain *C. calothyrsus* occurring in nearby fields, river margins and roadsides, albeit in generally small numbers. Their conservation in this system is somewhat difficult to predict, and is dependent on farmers continuing to cultivate and protect *C. calothyrsus*. One landowner at Santa María de Jesus who is aware of the importance of this population, allows seed to be collected from his trees and has agreed to maintain *C. calothyrsus* as coffee shade on part of his land (Chamberlain, 1994).

Populations found predominantly in the marginal land by roads are frequently disturbed by human activity, e.g., cutting, burning and road construction. Where the species is regularly cut, or after fire has spread through either deliberately or accidentally, the trees will often re-sprout and continue to reproduce. As with the bush fallow system described above, this type of land use will have an unpredictable impact on the genetic structure of the population and the number of individuals within it.

An interesting example of conservation is given by the roadside population at San Ramón, Nicaragua. Local people have been involved in, or are aware of, seed collecting activities surrounding a superior provenance of *Pinus tecunumanii* carried out by the national forest seed bank in the San Ramón area (Vilchez and Ravensbeck, 1992). The seed bank also collects seed from *C. calothyrsus*, which has given local people the impetus to propagate and protect *C. calothyrsus* on their land, and they now gain an income from this activity through the sale of seed. This is the exception to the rule, however, and it is likely that important resource populations of *C. calothyrsus* that occur predominantly in roadsides should also be conserved *ex situ* in order to maintain their genetic integrity and diversity.

Ex situ conservation

Ex situ collections of germplasm can serve an essential role in the conservation of material that may be difficult to conserve *in situ*, they may extend the range of variation protected and act as a reservoir for conservation activities in the future. *Ex situ* collections can also provide important monitoring functions. If collections are made regularly, samples can be genetically analysed by the use of molecular markers, and changes in the genetic make-up of the protected or important stands determined. Lastly, *ex situ* collections can serve as a source of material for further research, the results of which may allow improvements to be made to a conservation strategy.

Many kinds of *ex situ* conservation are possible including living plants, seeds in cold storage, tissue cultures and DNA in genomic libraries (Millar and Libby, 1991). The advantages and disadvantages of each of these approaches have been discussed in detail in the literature (Bonner, 1989; Hughes, 1998; Ledig, 1986; Millar, 1991). In the case of *C. calothyrsus*, consideration is given to two of these approaches; living material planted in botanic gardens and on research stations, and seed in medium- and long-term storage.

Living material

Ex situ conservation stands of *C. calothyrsus* may serve the dual purpose of the provision of seed for research, planting and sale. Given the economic value of *C. calothyrsus* and the difficulty in obtaining large quantities of superior seed (see Chapter 3), stands of the species planted outside its native range are likely to be established for seed production and serve a conservation role by default. In either case, however, similar considerations apply when establishing *ex situ* stands of *C. calothyrsus*.

Ex situ stands of *C. calothyrsus* are usually found in botanic gardens or the experimental stations of research institutes. For example, three seed production orchards established with seed from Patulul, San Ramón and Embu have been planted at an ICRAF experimental station at Embu, Kenya. Space can often be a problem when establishing *ex situ* stands, as adequate numbers of trees require large planting areas. Large numbers of trees will ensure that the variation in the native population is adequately represented and maintained in the *ex situ* stand, and for *C. calothyrsus*, large numbers of trees may also provide an attractive food resource for pollinators.

In order to maintain the genetic integrity of an *ex situ* conservation stand, it should be established with seed from only one native population (or provenance). The stand should be isolated from other sources of *Calliandra* pollen by several kilometres to avoid pollen contamination and the possibility of genetic erosion. Some seed production areas are being planted with mixtures of populations, e.g., a seed orchard planted in Hawaii contains trees established with seed from San Ramón and La Puerta, Nicaragua (A.J. Pottinger, pers. comm.[3]). Other orchards representing separate populations may have been planted too close together with the possibility for contamination between them being high. These types of seed orchards can not, therefore, be considered as *ex situ* conservation stands.

Because of the emphasis on seed production, *ex situ* conservation stands have been, and will be, established with seed from the most productive populations in terms of growth and biomass accumulation. Three *ex situ* seed production areas have been established in the native range that could be used for conservation purposes, and have been established with seed from La Ceiba, Honduras (Lancetilla Botanic Gardens), Fortuna, Costa Rica (ICTA) and Santa María de Jesus, Guatemala (Trees, Water and People).

Seed storage

Seed of *C. calothyrsus* is hard-coated (orthodox) and can be stored at 4ºC for periods of over five years with germination percentages of 75-90% (see section 4.1). The OFI seed collections of *C. calothyrsus* are maintained at the germplasm bank of the International Centre for Research in Agroforestry (ICRAF), Nairobi, Kenya. The seed is stored at a temperature of 4ºC and sealed in foil bags maintained in air tight metal tins which protect the seed from external changes in humidity and prevent insect and fungal attack (I. Dawson, pers. comm.[4]). Although not specifically maintained for conservation, the seed collection at ICRAF does provide a reservoir of germplasm that can

be used for conservation purposes, e.g., the propagation of seed as living plants, or the transfer of seed to long-term storage.

As part of a strategy to ensure the conservation of core populations of OFI's collection of tropical pine and woody legume seed, eight accessions of *C. calothyrsus* and two of *C. houstoniana* were deposited in the long-term seed storage facility at the Royal Botanic Gardens at Wakehurst Place, Sussex, UK (Pottinger, 1996a). Between 1500 and 3000 seeds per seedlot were dried to 10% relative humidity at 20ºC, sealed in a foil bag and maintained at -20ºC. The seedlots stored in this way are listed in Table 8.5 and will remain in storage over a period of decades. The seedlots were selected on the basis of the growth performance of *C. calothyrsus* across a series of trials and the availability of seed in sufficient quantities at the time.

Table 8.5:   Seed in long-term storage at the Royal Botanic Gardens, Wakehurst Place, Sussex, UK (Pottinger, 1996a).

Species	Population
C. calothyrsus	
C. calothyrsus-1	Flores, Guatemala
	Patulul, Guatemala
	Alotenango, Guatemala
	Barillas, Guatemala
	Bonampak, Mexico
	La Ceiba, Honduras
	Bonito Oriental, Honduras
C. calothyrsus-3	San Ramón, Nicaragua
C. houstoniana	Minatitlan, Mexico
	Tuxtepec, Mexico

The Desert Legume Program of the US National Plant Germplasm System in Tuscon, Arizona has eight taxa of *Calliandra* represented by 37 accessions in its long-term seed storage facility (M.B. Johnson, pers. comm.[5]), although none of these taxa is *C. calothyrsus*.

8.3   Current conservation needs

Current conservation measures for *C. calothyrsus* ensure that populations from the subgroups *C. calothyrsus-1* to *-3* are represented in *ex situ* seed

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[3] CNRD, Green College, University of Oxford, Woodstock Road, Oxford, UK
[4] ICRAF, P.O. Box 30677, Nairobi, Kenya

[5] The University of Arizona, 2120 East Allen Road, Tucson, Arizona 85719, USA

stands or long-term seed storage, or a mixture of both (see Table 8.3). At least one population from each of the subgroups *C. calothyrsus-1* to *-3* are also reasonably secure *in situ*. However, populations representing *C. calothyrsus-4*, a taxon that has performed poorly in biomass trials and hence has little economic value, have not until now, been identified for conservation concern. Priority should be given to the inclusion of this taxon into conservation initiatives for the species, along with a number of other important resource populations that are, as yet, unrepresented in *ex situ* stands or long-term seed storage, or which may be at risk *in situ*.

Future conservation initiatives for *C. calothyrsus* may include:

In situ conservation: There are plans to link the remaining natural habitats in Central America with national parks, biological reserves and zones of agroforestry, or forest plantation (Hogan, 1998). The aim is to form a biological corridor across the length of the isthmus, and foster conservation initiatives between politicians, social and natural scientists, and indigenous rights activists. If successful, this enterprise may serve to protect *in situ* existing populations of *C. calothyrsus*. *Circa situm* conservation of *C. calothyrsus* has potential through use of the species as coffee shade, and perhaps its occurrence as a component of bush fallow in agricultural land or by roadsides. The continued 'use' of *C. calothyrsus* in both these systems may be difficult to predict, however, particularly given the low status of the tree in the native range, and potential changes in land use.

Ex situ conservation: Seed of the more vulnerable *C. calothyrsus-4* populations needs to be added to the seed collections in long-term storage. It would also be appropriate to have one or two populations representing *C. calothyrsus-2* given this taxon's occurrence only in Costa Rica and Panama. The planting of *ex situ* seed production orchards of the populations listed in Table 8.3 should also be given high priority to facilitate the supply of productive seed, whilst ensuring the conservation of important resource populations.

Conservation status of *C. calothyrsus*

- *C. calothyrsus* is a widespread, sometimes locally abundant species at low risk from extinction.

- Intra-specific diversity in the species, and the economic importance of some of its populations means, however, that conservation of *C. calothyrsus* is merited.

- Units of conservation can be diagnosed at two levels: (1) the four distinct sub-groups within the species (*C. calothyrsus-1* to *-4*); and (2) specific populations of economic importance.

- 10% of surveyed populations are found in biological reserves and can be secured through *in situ* conservation.

- 46% of surveyed populations are found in riparian habitats and may be relatively secure from extinction.

- Remaining populations are found cultivated as coffee shade, or occur as bush fallow in agricultural land or by roadsides. The security of these populations may be difficult to predict as they are particularly vulnerable to human disturbance.

- Populations representing *C. calothyrsus-4* are most vulnerable to extinction due to their narrow distribution, and occurrence as bush fallow remnants of often only a few individuals.

Plate 6

A - Hedgerow inter-cropping of *Calliandra calothyrsus* with maize in Indonesia.

B - A natural roadside population of *Calliandra calothyrsus* at San Ramón, Nicaragua.

C - A roadside tree of *Calliandra calothyrsus* at Copán, Honduras.

D - *Calliandra calothyrsus* occurring on the banks of the River Cangrejal, La Ceiba, Honduras.

E - Swathes of *Calliandra calothyrsus* colonising the banks of the River Cangrejal, La Ceiba.

F - *Calliandra calothyrsus* occurring in the mountain village of Barillas, Guatemala.

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