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FORESTRY RESEARCH PROGRAMME

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Genetic improvement of *Calliandra calothyrsus* - phase II

R6535 - Final Technical Report

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Executive Summary

Over the last ten years or so there has been substantial interest in cultivating *Calliandra calothyrsus* in the humid tropics, particularly on acid soils where other agroforestry species tend to grow poorly. As a result, the species is now cultivated for practical or research purposes in 40 countries world-wide.

With the widespread cultivation of calliandra and the identification of superior seed sources by the International Calliandra Trial Network, the need to produce local sources of good quality seed has increased. Many researchers and field workers have had, however, poor experiences with seed production from calliandra. Low seed set per tree has been a common occurrence in many seed stands planted outside the native range. This has been the driving force for recent research on the reproductive biology of calliandra and the factors that control seed production in the species.

The purpose of this project was to lay sound foundations for the future use and improvement of the best seed sources of *Calliandra calothyrsus*. The project therefore focused on achieving the following outputs:

1. Family-based seed collections of provenances identified as having superior growth performance will be incorporated into improvement work.

2. Seed orchard design and seed production optimised through increased knowledge of the reproductive biology, pollination system and gene flow in the species.

3. The extent of inter-specific hybridisation within natural populations investigated, and its potential for tree improvement assessed.

4. Important resource populations that are under threat, or of low genetic diversity, will be identified for conservation. Combined with the results from the breeding system and hybridisation experiments, a rational conservation strategy for the species will be developed.

5. A synthesis of the state of knowledge surrounding *C. calothyrsus* published in the form of a Tropical Forestry Paper (TFP).

6. The potential for pollen contamination between seed orchards of *C. calothyrsus* determined.

Fieldwork was carried out in Central America, Mexico, Kenya and Indonesia and contributed to the achievement of the following results:

1. Provenance/progeny trials established in Honduras and Guatemala were evaluated and the results used in the International *Calliandra* Trial Network.
2. Seed production orchards have been established in collaboration with a number of organisations. Three orchards have been established by ICRAF and KARI at Embu, Kenya, a fourth orchard has been established in collaboration with the North American NGO, New Forests, on community land near Escuintla, Guatemala, and the trials established in Honduras and Guatemala will be converted to seed orchards.
3. Information on the reproductive biology of calliandra (flowering and fruiting, breeding system, sexual system, pollination system, gene flow within populations and potential for inter-specific hybridisation) has been assembled and analysed. This work is presented in a monograph on *C. calothyrsus*, OFI Tropical Forestry Paper No.40, and the implications for seed production presented in a field manual for extension workers and researchers.
4. *C. calothyrsus* is a widespread, sometimes locally abundant species at low risk from extinction. However, the high level of intra-specific diversity in the species, and the economic importance of some of its populations means that conservation of *C. calothyrsus* is merited. A strategy for the conservation of variability in the species is presented with populations representing *C. calothyrsus-4* being most vulnerable to extinction.
5. The potential for pollen contamination between seed orchards of *C. calothyrsus* planted within an isolation distance to 2 km between orchards was found to be low. However, differences between orchards in flowering time and the quantity of flowers produced may increase this potential.

Throughout the humid tropics, there is continued interest in obtaining or cultivating improved material of *C. calothyrsus,* particularly on sites with acid soils. Orders for seed currently outstrip supply from the native range, but through various seed production initiatives, principally in Kenya and Indonesia, this demand is being addressed. Furthermore, the dissemination outputs of the project will allow users of the species access to up-to-date information on the species.

Acknowledgements

The extensive amount of time dedicated to fieldwork undertaken during this project required substantial logistic support, and it is through the collaboration of many organisations and individuals that this project has been able to achieve its goals.

In Guatemala, the collaboration of ICTA, BANSEFOR and DIGEBOS is gratefully acknowledged. The official agreement between OFI and the latter organisations has greatly facilitated the work, but special thanks go to Rodrigo Arias and Carlos Rodriguez at ICTA who are acknowledged for their advice, considerable logistic support and the establishment of trials.

In Honduras, support from CONSEFORH, COHDEFOR, and ESNACIFOR through the Forest Seed Bank and Lancetilla Botanic Gardens has been of enormous value. Special thanks are due to Ernesto Ponce, and Kevin Crockford from the former organisation for their advice, generous hospitality, and considerable logistic support. The establishment of a trial at Lancetilla Botanic Gardens has been conducted in collaboration ESNACIFOR and CONSEFORH staff whose work is gratefully acknowledged. The assistance of Oscar Leveron at the seed bank in ESNACIFOR, Manuel Hernandez-Paz, Director of ESNACIFOR, and Cero Navarro, Romulo, Armando and Pepe Montesinos at Lancetilla has also been greatly appreciated.

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Fieldwork in Central America was carried out with the assistance of Alcuin Arkotza, Douglas Gibbs, Gus Hellier, Jason Hubert, Andrew Matthews, John Roberts and David Southall who along with Modesto Castillo Sanchez deserve particular thanks for all their hard work.

Thanks are due for all the hard work undertaken in storage, testing and dispatch of seed from the Forestry Commission Research Station at Alice Holt.

At OFI and CNRD, support has come from many quarters and thanks go out to all staff. I would like to thank Alan Pottinger for managing the *Calliandra* trial network and his invaluable help and advice; Andrew Dunsdon for assistance with statistics; Christine Surman for all her hard work dealing with seed and herbarium specimens; Martin Billingham, David Boshier, Stephen Harris, and Jason Hubert for support and advice; and Sandie Hardaker and Chris Hawes for administrative help.

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Acronyms

CATIE	Centro Agronómico Tropical de Investigación y Enseñanza, Turrialba, Costa Rica
CIAT	Centro Internacional de Agricultura Tropical, Cali, Colombia
CNRD	Centre for Natural Resources and Development, Green College, UK
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, Switzerland
KARI	Kenya Agricultural Research Insitute, Kenya
KEFRI	Kenya Forestry Research Institute, Kenya
KWDP	Kenya Woodfuel Development Programme, Kenya
NifTAL	Nitrogen Fixation for Tropical Agriculture, University of Hawaii, USA
NRC	National Research Council, Washington D.C., USA
OFI	Oxford Forestry Institute, UK

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Background

Calliandra calothyrsus Meisn. (Leguminosae - Mimosoideae) is a small woody legume that is distributed widely throughout Central America and southern Mexico. The species occurs across a variety of ecological conditions from very wet (over 4000 mm rainfall per year) to seasonally dry (as low as 700 mm rainfall per year), and from sea-level to 1800 m altitude in southern Guatemala. *C. calothyrsus* is a riverine coloniser in natural undisturbed forest, but is now a common element of secondary vegetation in areas such as roadsides and fallow agricultural land. It often appears in thickets during early colonisation, but with time tends to be out-competed and has a scattered distribution in older stands. The species is bat-pollinated and assumed to be outcrossing.

Calliandra calothyrsus has been cultivated and used widely for the provision of fuelwood, animal fodder, green manure, shade and soil conservation. The species was introduced in the 1930's from Guatemala to Indonesia and is now naturalised in many parts of Java. Seed from these naturalised populations has been, until recently, the major source of material for subsequent planting in south-east Asia, Australia and Africa. In 1990, a DFID-funded Forestry Research Programme (FRP) project (R4585) was initiated at the Oxford Forestry Institute (OFI) to map the natural distribution of *C. calothyrsus* and collect seed for an international network of provenance trials that aimed to determine the best seed sources for biomass production. It is anticipated that demand for seed of the best provenances will be great once the final results are obtained by trial collaborators. The distribution and use of selected material by researchers, NGO's and farmers will then be dependent on the ability to produce large quantities of seed. Seed production in the native range is, however, limited by both the small size of *C. calothyrsus* populations and low seed set per tree.

In order to improve seed production in *C. calothyrsus*, and anticipate demand for seed of the best provenances, seed orchards were established under FRP research project R5728 in both the native range, and where the species is cultivated. Low seed production has, however, often been obtained in orchards established in exotic environments (Boland and Owour, 1996; B.M. Kamondo, pers. comm.[1]; B. Duguma, pers. comm.[2]). *C. calothyrsus* flowers precociously, but sets relatively little seed in comparison to the abundance of flowers it produces, and hence, there has been the perception that an individual tree should produce more seed. Exceptionally low seed production has, however, been obtained in some cases, and there may be one or more of a range of factors responsible for this including the species' breeding and/or sexual system, low visitation rates by pollinators, or adverse climatic conditions. This report summarises the completion of research, initiated under R5728, on the reproductive biology and genetic diversity of *C. calothyrsus* and the factors controlling seed production in the species.

[1] Kenya Forest Research Institute, P.O. Box 20412, Nairobi, Kenya
[2] IRA/ICRAF Project, P.B. 2067, Yaounde, Cameroon

Project Purpose

Germplasm of multipurpose tree species secured and improved

The overall aim of the project was to lay sound foundations for the future use and improvement of the best seed sources of *Calliandra calothyrsus*. The outputs of the project, which had been partially fulfilled under R5728 were as follows:

1. Family-based seed collections of provenances identified as having superior growth performance will be incorporated into improvement work.

2. Seed orchard design and seed production optimised through increased knowledge of the reproductive biology, pollination system and gene flow in the species.

3. The extent of inter-specific hybridisation within natural populations investigated, and its potential for tree improvement assessed.

4. Important resource populations that are under threat, or of low genetic diversity, will be identified for conservation. Combined with the results from the breeding system and hybridisation experiments, a rational conservation strategy for the species will be developed.

5. A synthesis of the state of knowledge surrounding *C. calothyrsus* published in the form of a Tropical Forestry Paper (TFP).

6. The potential for pollen contamination between seed orchards of *C. calothyrsus* determined.

Outputs

1. Family-based seed collections of provenances identified as having superior growth performance will be incorporated into improvement work.

Status: ***Achieved***

The project has either funded directly, or collaborated in funding, the establishment of a number of **seed production orchards**. This has involved provenance and progeny testing, as well as the establishment of orchards with bulk seed collections of individual provenances.

1.1. Provenance trial at Cuyuta, Guatemala

1.1.1. Background and methods

A trial was established within a one hectare plot, fenced off to prevent animals grazing within it at Cuyuta, the experimental station of ICTA, Guatemala. The trial contained four provenances of *C. calothyrsus* established with seed from Santa María de Jesus, Guatemala (33/93), Patulul, Guatemala (9/91), Barillas, Guatemala (35/93), and Fortuna, Costa Rica (108/94), in a randomised block design. The blocks form 12 replications, and the families within each block were arranged as 6-tree line plots at a spacing of 3m x 0.5m. The seed for the trial was obtained from the OFI *Calliandra* seed collection. The seedlings were raised in the nursery at Cuyuta and planted in August 1995.

The trial was assessed in 1996 and 1997, and the following traits were measured: height of the main stem (m), number of stems, diameter of the stems 30cm above ground (cm), and wood biomass (kg). The biomass assessment was only carried out in 1997 and the trial was systematically thinned by 50%.

1.1.2. Results

The provenance from Fortuna was found to be the most productive wood producer of all the provenances tested, a result that was consistent across the two years of assessment (Figure 1.1).

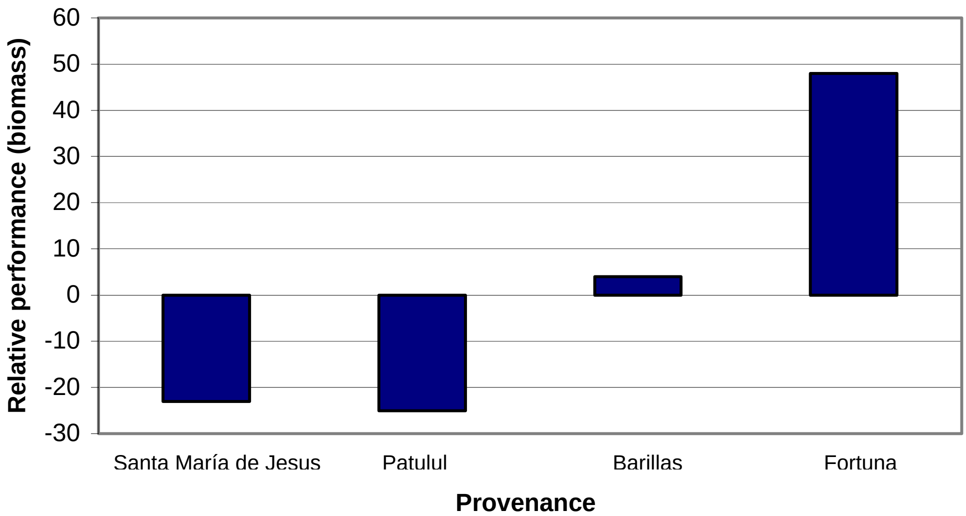

Figure 1.1 Wood production: mean relative performance of four provenances of *Calliandra calothyrsus* grown at Cuyuta, Guatemala in 1997.

After the third assessment, conversion of the trial to a seed production area will be considered. It has been recommended, on the basis of the results so far, that the provenances from Santa María de Jesus, Patulul and Barillas be removed to leave only the trees from Fortuna. Fortuna was an above average performing provenance in a number of other trials (Pottinger and Dunsdon, 2001), but is locally threatened in its native range. The population is scattered across a wide area within a river, fallow agricultural land and roadsides. Trees in the latter areas are repeatedly cut back each year, do not appear to produce seed and may contribute minimally to the pollen pool. Hence, the *ex-situ* conservation of this provenance is of particular priority. The conversion of the trial at Cuyuta to a seed production area of Fortuna would be an important contribution to the conservation of *C. calothyrsus*.

1.2. Progeny trial at Lancetilla Botanic Gardens, Honduras

1.2.1. Backgrounds and methods

The trial was established within a one hectare plot, fenced off to prevent animals grazing within it at Lancetilla Botanic Gardens, Honduras. The trial contained 30 families of *C. calothyrsus* established with seed from La Ceiba, Atlantida, in a randomised block design. The blocks form 29 replications, and the families within each block were arranged as 6-tree line plots at a spacing of 3m x 0.5m. The seed for the trial was obtained from the OFI *Calliandra* seed collection, and from seed collected by the CONSEFORH project, Siguatepeque, in 1994. The seedlings were raised in the nursery at Lancetilla and planted in August 1995 with the assistance of staff from CONSEFORH.

The trial was assessed in 1996 and 1997, and the following traits were measured: height of the main stem (m), number of stems, diameter of the stems (cm) 30cm above ground and wood biomass (kg). The biomass assessment was only carried out in 1997 and the trial was systematically thinned by 50%.

1.2.2. Results

Families 2, 4, 5, 8, 9, 14, 15, 18, 20, 22, 25 and 27-30 were the most productive wood producers in the provenance from La Ceiba across the two years of assessment in a progeny trial at Lancetilla Botanic Gardens (Figure 1.2). Growth rates of the main stem were found to decrease after the first six months, but stem number was found to increase. After the third assessment, conversion of the trial to a seed production area was done. The poor performing families were removed to leave only the trees from the above families. With the number of replications in this trial, it is likely that diversity can be maintained whilst maximising the gain in productivity.

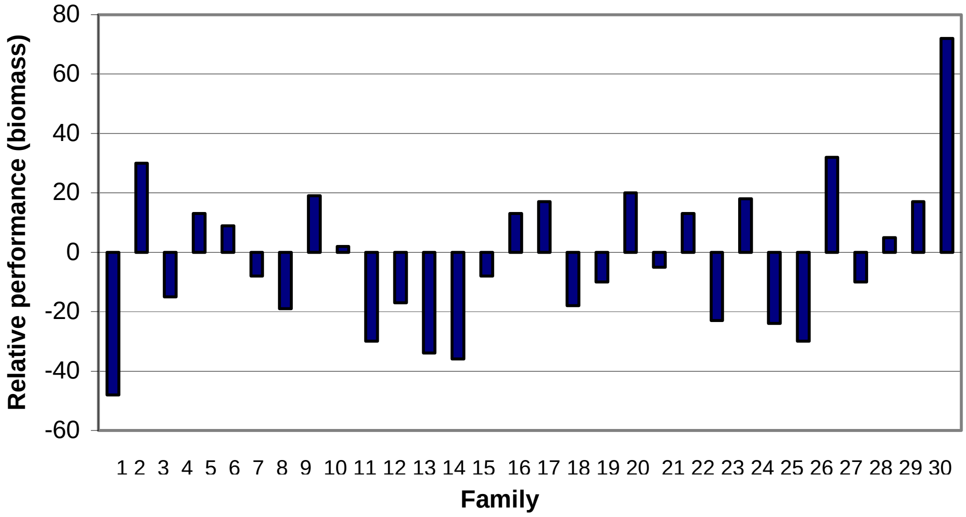

Figure 1.2: Wood production: mean relative performance of 30 families of *Calliandra calothyrsus* from La Ceiba, Honduras, grown within a trial at Lancetilla Botanic Gardens in 1997.

1.3. Seed orchards established with bulk seed collections

1.3.1. Background and methods

Four seed production orchards have been established in collaboration with R6535. Three orchards have been established by ICRAF and KARI at Embu, Kenya, and comprise trees established with seed from Patulul (Guatemala), San Ramón (Nicaragua) and Embu (Kenya) with each provenance being represented in a separate orchard of approximately 0.25 ha. The fourth orchard has been established in collaboration with the North American NGO, New Forests, on community land near Escuintla, Guatemala. The orchard comprises trees established with seed from Santa María de Jesus (Guatemala).

For further information, refer to:

CHAMBERLAIN, J.R. (2001). Conservation. pp. 77-85. In: *Calliandra calothyrsus*, an agroforestry tree for the humid tropics (Ed.) Chamberlain, J.R. Tropical Forestry Paper 40, Oxford Forestry Institute, Oxford, UK.

POTTINGER, A.J. and DUNSDON, A.J. (2001). Provenance trials. pp. 53-66. In: *Calliandra calothyrsus*, an agroforestry tree for the humid tropics (Ed.) Chamberlain, J.R. Tropical Forestry Paper 40, Oxford Forestry Institute, Oxford, UK.

2. Seed orchard design and seed production optimised through increased knowledge of the reproductive biology, pollination system and gene flow in the species.

Status: *Achieved*

A thorough understanding of reproductive biology is critical for the effective conservation, management and utilisation of a plant species. Studies on reproductive biology involve floral morphology and phenology, the sexual, breeding and pollination systems, fructal phenology and seed dispersal. **Floral phenology** is fundamental to understanding the genetic structure of populations and to ensuring the production of seed for afforestation. Variation in patterns of flowering may represent a means by which a species can adapt to its environment through plant-pollinator interactions, or seasonal changes in water availability. For outcrossing species, the flowering of individuals relative to one another and to their pollinators will affect patterns of mating and gene flow. The **breeding system** of a plant species, i.e., whether the species is outcrossing or self-fertile, will influence the partitioning of genetic variation within and between populations, which will in turn affect the choice of sampling strategies for seed collection, and the design of seed production orchards. The type of **pollinator** and their mode of behaviour when foraging for pollen and nectar resources will similarly affect the genetic structure of populations, and may influence the plant species' adaptation to exotic environments. Lastly, **fructal phenology** and the mode of seed dispersal will ensure the production and distribution of seed from the mother tree, the pattern of which will again affect gene flow and population genetic structure.

Studies on the reproductive biology of *C. calothyrsus* have a special significance because low seed production yields have often been obtained where the species is cultivated in exotic environments. There may be one or more of a range of factors responsible for this including low visitation rates by pollinators and adverse climatic conditions, as well as the species' reproductive biology. The following sections summarise the research on the floral morphology and phenology, and the sexual, breeding and pollination systems of *C. calothyrsus*. Their implications for improved seed production and seed orchard design are discussed, and a summary of the main findings and their implications is presented at the end of each section.

2.1. Floral morphology and phenology

2.1.1. Background and methods

Studies on the floral morphology of *C. calothyrsus* have included measurements of the species':

- floral structure
- floral anthesis
- stigma receptivity
- nectar production and sugar concentration
- pollen production
- phenology of an individual flower

Phenological observations were made within a natural population at Bonito Oriental, Honduras, and information on flowering and fruiting phenology across the native range was collated to gain an overall picture of the flowering phenology in the species. The Bonito Oriental 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.

2.1.2. Results

2.1.2.1. Floral morphology: The inflorescence of *C. calothyrsus* is a paniculate raceme with flowers that open acropetally 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 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 outwards and downward to reveal the polyads which are eight-grained aggregates of pollen grains. The style is white to pale yellow and 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. Anthesis occurs between 15:30 and 17:00 h, although in Kenya was recorded to occur as early as 14:00 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 been lost and the flowers begin to wilt. The number of flowers produced per inflorescence per day has been variously recorded as 1-34 with some trees regularly producing higher numbers of flowers. The standing volume of nectar has been recorded within the range of 20-50 µl per flower with sugar concentrations in the range 14-20%. From pollination to seed maturity, a period of between 90-120 days is required.

2.1.2.2. 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 to up to twelve months per year if sufficient moisture is available, but is concentrated between October and January, a period after the main wet season but before the onset of the dry season in Mesoamerica. 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. At Bonito Oriental, Honduras, 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.

Summary
Flower production 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 been lost 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* corresponds to the wet season in both native and exotic environments.
- There is evidence for provenance variation floral phenology and flower number that may be influenced by adaptation to local climatic conditions, and the allocation of resources.

Implications: Pollination will only be effected by nocturnal visitors to the flowers. Peak flowering will occur during the wet season, allowing seed to ripen successfully during the subsequent dry season. There may be provenance variation for seed production, hence the potential for greater seed production from some provenances.

2.2. Sexual system

2.2.1. Background and methods

Calliandra calothyrsus is **andromonoecious**, i.e. individual trees bear both hermaphrodite (bisexual) and staminate (functionally male) flowers. Staminate flowers, when produced, have been observed to occur either at distal positions on the inflorescence, or when the pods are developing and then the staminate flowers will occur in a medial position on the inflorescence. The staminate flowers are distinguished at anthesis by their completely reduced styles, or withered style remains, and cannot be pollinated or go on to produce seed. In terms of the male function, i.e. the production of fertile pollen, however, the staminate flowers are perfectly functional.

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 male and female function. 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 trial plot of *C. calothyrsus* at the National Forestry School's (ESNACIFOR) Experimental Station, San Juan, Siguatepeque, Honduras during 1994 and 1995. The production of hermaphrodite flowers, staminate flowers and fruits were monitored over a period of 28 days. Within a further two 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 fruit (unpollinated), whilst the remaining half were allowed to set fruit as normal (pollinated), and (b) half the inflorescences on an individual tree did not set fruit, whilst the other half were allowed to set fruit as normal. In this way, the plasticity of sex expression could be investigated at the between-tree and within-tree levels.

2.2.2. Results

In all 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 2.1a and 2.1b). 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, 2000). 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*.

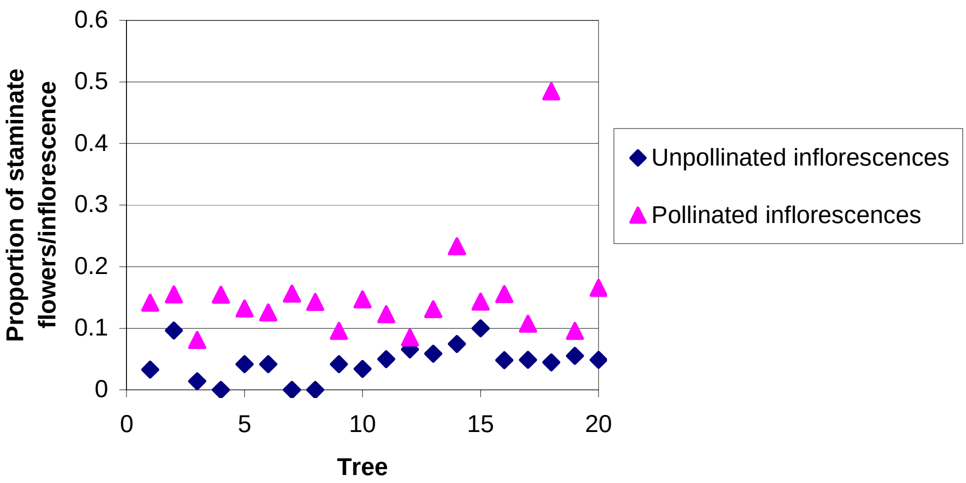

Figure 2.1a: Mean proportion of staminate flowers per inflorescence produced by each of 20 trees in pollinated (plus fruit) and unpollinated (no fruit) treatments during an investigation of whole plant sex expression

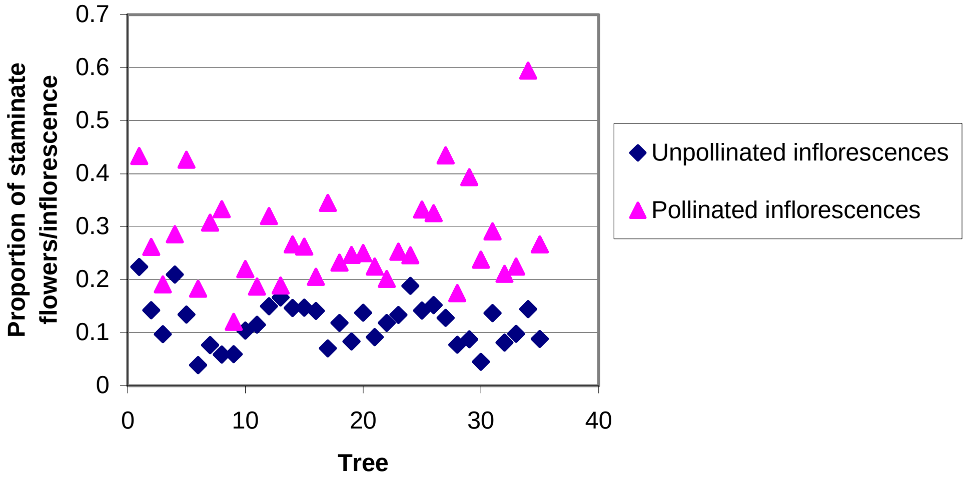

Figure 2.1b: Mean proportion of staminate flowers per inflorescence produced by 35 trees in pollinated (plus fruit) and unpollinated (no fruit) treatments during an investigation of inflorescence sex expression.

Summary
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.

2.3. Breeding system

2.3.1. Background and methods

Calliandra calothyrsus was **assumed to be 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 style and stamen length, and (4) its pollination by bats and large moths. This hypothesis was tested with the use of a series of experiments involving controlled pollinations, and the species' mechanism of self-incompatibility was determined.

2.3.1.1. Controlled pollinations: The breeding system of *C. calothyrsus* was studied using controlled pollinations on trees selected from a number of trial plots: (1) at Cuyuta, the Experimental Station of ICTA (Instituto de Ciencia y Tecnologia Agricolas), Guatemala, and (2) at Lancetilla Botanic Gardens, Honduras. A standard pollinating procedure was used in which the inflorescences chosen were bagged once the flowers began to open, and any previously fertilised flowers removed. The flowers were then emasculated prior to anther dehiscence and re-bagged. Pollen was collected from selected trees and applied with a fine brush to receptive stigmas from 19:30 h onwards. All flowers on an inflorescence received the same type of pollen. A number of inflorescences on the same trees were tagged and left unbagged to monitor the amount of successful fertilisation (fruit set) under natural conditions. Emasculated and pollinated flowers were also fixed after 12 to 84 hours, and the fixed material was later examined with fluorescent microscopy to determine the mechanism of self-incompatibility.
2.3.1.2. *Mechanism of self-incompatibility*: To determine the mechanism of self-incompatibility in *C. calothyrsus*, controlled pollinations were carried out according the regime described above. Whole pistils were removed from the treated flowers and fixed for 1 hour in acetic acid and ethanol, 3:1 by volume. The fixed pistils were then transferred to 70% ethanol and stored at room temperature until examination could take place. The pistils were softened and cleared in 8M sodium hydroxide overnight and rinsed in distilled water for 48 hours prior to staining. Pistils were squashed in decolourised aniline blue on a microscope slide and examined using a Zeiss epifluorescence microscope. Pollen tubes were located by the blue-green fluorescence of the callose polysaccharide component of their walls and plugs.

2.3.2. Results

2.3.2.1. Controlled pollinations: Table 2.1 summarises the results of the controlled pollination experiments in *C. calothyrsus* carried out to investigate the breeding system of the species. The percentage fruit set from open-pollinated plants was similar in both experiments. Crosses within a particular provenance were generally more successful than crosses between provenances. The percentage fruit set from self-pollinated plants of *C. calothyrsus* was very different in the two experiments. Percentage fruit set on selfing at Cuyuta (mean = 4.42, SE = 0.89) was similar to that found in a hybridisation experiment carried out in Honduras (mean percentage fruit set = 2.10, SE = 1.08) and in an experiment carried out in Sri Lanka (mean = 2.66, S.E. = 2.06; Rajaselvam and Chamberlain, 1996). In the experiment at Lancetilla, however, fruit set was significantly greater with a 10-fold difference in the number of fruits set after self-fertilisation.

Table 2.1: Comparison of the results from two breeding system experiments carried in Guatemala and Honduras during 1995 and 1996 (standard errors in parentheses).

Cross	Mean percentage fruit set	Mean seed ovule ratio
Cuyuta, Guatemala		
Selfing	4.42 (0.89)	0.37 (0.09)
Within-provenance	26.04 (3.00)	0.54 (0.02)
Between -provenance	15.69 (2.33)	0.51 (0.01)
Open pollination	15.06 (1.70)	0.56 (0.02)
Lancetilla, Honduras		
Selfing	32.39 (4.05)	0.65 (0.02)
Within-family	35.06 (4.46)	0.69 (0.02)
Between-family	44.61 (3.49)	0.75 (0.01)
Open-pollination	17.09 (2.13)	0.62 (0.02)

This result, in the light of previous experiments, was thought to be rather unusual, and was investigated the following year (1997) with further controlled pollinations. Due to the small number of flowers available in the trial plot, all the pollinations not aborted up to 84 hours after pollination were harvested (30.63% of the flowers selfed), and examined with fluorescent microscopy to determine the number of ovules penetrated per ovary. Ovule penetration on self-fertilisation was found to be poor when compared to that from cross-fertilisation, and suggested that the percentage fruit set from selfing would have been lower in 1997 than that obtained in 1996.

These results suggest that provenance incompatibility barriers may be varied in *C. calothyrsus*, and incompatibility can vary with the age of the plant. When the first experiment took place at Lancetilla in 1996, the plants were only seven months old, but flowered and set seed profusely. The ability to tolerate selfing whilst juvenile has been demonstrated for colonising plants, and is hypothesised to confer an advantage on a population if there are only a few founder individuals.

2.3.2.2. Mechanism of self-incompatibility: Microscope observations of the pollinated pistils showed that pollen grains germinated readily on the stigma following both cross- and self-pollination. After 18-21 h, pollen tubes resulting from both cross- and self-pollination had reached the base of the style and could be observed in the ovary, suggesting that the self-incompatibility system in *C. calothyrsus* is gametophytic and late-acting. Self pollen was found to penetrate the ovules in the ovary, but with reduced success when compared to cross pollen (Figure 2.2). 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.

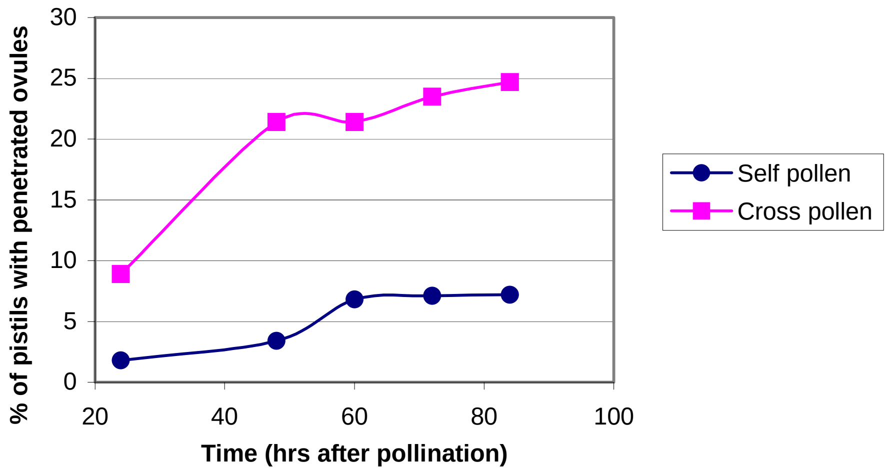

Figure 2.2: Percentage of pistils with penetrated ovules resulting from cross- and self-pollination when examined at different time intervals after pollination.

Summary
Breeding system of *C. calothyrsus* and its implications for seed production

- *Calliandra calothyrsus* is predominantly outcrossing, but in possession of a weak, 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 a greater tolerance of selfing early in the life-history of *C. calothyrsus*.

Implications: There is evidence that *C. calothyrsus* possesses a mixed mating system and the degree of selfing in *C. calothyrsus* 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 maintained through ensuring that the seed used for establishment has come from a broad genetic base.

2.4. Pollination system and gene flow

2.4.1. Background and methods

Detailed observations on the **pollinators** of *C. calothyrsus* and their foraging behaviour were carried out through capture, mark and recapture experiments within a river valley in Honduras, and the results have been described in detail elsewhere (Chamberlain, 1996). To assess the degree of **outcrossing** and extent of **gene flow** in *C. calothyrsus*, seed was collected from 44 individual trees in five sub-populations within the same river valley population. Isozyme electrophoresis was employed to examine 20 progeny from each tree in each sub-population, and for the population as a whole. 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. Paternity exclusion analysis (Devlin and Ellstrand, 1990) was employed to estimate pollen flow.

2.4.2. Results

2.4.2.1. Outcrossing rate: 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 2.2. 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.

Table 2.2: 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).

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)**

2.4.2.2. Pollen-mediated gene flow: 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 2.3). 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.

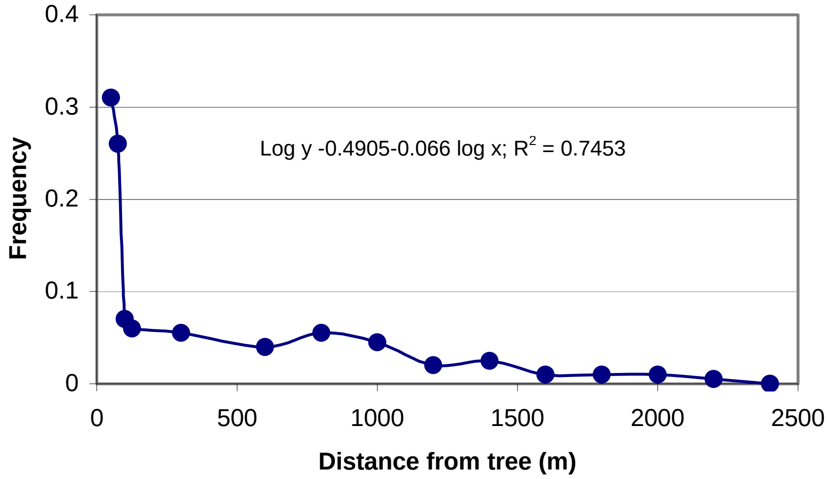

Figure 2.3: Pollen flow distances in *Calliandra calothyrsus* in the River Bonito, Colón, Honduras.

Summary
Mating system and gene flow in *C. calothyrsus* and its implications for seed production

- 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.

For further information, refer to:

CHAMBERLAIN, J.R. (1998). *Calliandra calothyrsus*: Reproductive biology in relation to its role as an important multipurpose tree. pp. 439-448. In: Reproductive Biology in Systematics, Conservation and Economic Botany. (Eds.) Owens, S.J. and Rudall, P.J. Royal Botanic Gardens, Kew.

CHAMBERLAIN, J.R. (2000). Improving seed production in *Calliandra calothyrsus*: a field manual for researchers and extension workers. Miscellaneous Paper, Oxford Forestry Institute, Oxford, UK. 37 pp. English, Indonesian and Spanish.

CHAMBERLAIN, J.R. Sex expression in the andromonoecious mimosoid legume, *Calliandra calothyrsus* (Leguminosae). Paper in press with the *Canadian Journal of Botany*.

CHAMBERLAIN, J.R. and HUBERT, J.D. (2001). Reproductive biology and seed production. pp. 13-28. In: *Calliandra calothyrsus*, an agroforestry tree for the humid tropics. (Ed.) Chamberlain, J.R. Tropical Forestry Paper 40, Oxford Forestry Institute, Oxford, UK.

CHAMBERLAIN, J.R. and HUBERT, J.D. (2001). Molecular analysis of genetic variation. pp. 67-76. In: *Calliandra calothyrsus*, an agroforestry tree for the humid tropics. (Ed.) Chamberlain, J.R. Tropical Forestry Paper 40, Oxford Forestry Institute, Oxford, UK.

3. The extent of inter-specific hybridisation within natural populations investigated, and its potential for tree improvement assessed.

Status: *Achieved*

3.1. Natural hybridisation

3.1.1. Background and methods

Interspecific hybridisation between *Calliandra* series *Racemosae* was suspected to occur naturally between *C. calothyrsus* and *C. houstoniana* (in Copán, Honduras; Nuevo Ocotepeque, Honduras; Meambar, Honduras; Chicosan, Mexico; and Flores, Guatemala), and for *C. houstoniana* and *C. juzepczukii* in Mexico (Ocozocautla, Chiapas). If speciation has occurred in the past through a process of ecological isolation rather than through reproductive isolation, habitat disturbance following human activity, e.g., agriculture, road construction, etc., in combination with the pioneer nature of many *Calliandra* species, may lead to hybridisation between previously separated species. Hybridisation is associated, however, with the possible loss of valuable provenances for seed collection, and taxonomic confusion may result. 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 could be threatened (Dawson *et al.*, 1996).

Field studies at five sites where sympatric populations of *C. calothyrsus* and *C. houstoniana* had been identified were undertaken in 1995. For all the sites, with the exception of Flores, Guatemala, no putative hybrids could be identified, despite an overlap in the individual species' flowering times. In Flores, 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, e.g., one specimen produced flower buds, but without their floral and reproductive organs. In general, pod production was very low on the intermediate types compared to the neighbouring true species, but growth may have been more vigorous. 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.*

Vegetative samples of *C. calothyrsus*, *C. houstoniana* subsp. *houstoniana*, and the intermediate form were collected from two locations near Flores to determine the identity of the intermediate form. Fifteen morphological traits were measured from mature, fresh material and averaged for each specimen and morphometric analyses conducted, i.e., character count and principal components analyses. The leaf material was also analysed using randomly amplified polymorphic DNA markers (RAPDs).

3.1.2. Results

The analysis of morphological traits (through character count and principal components analysis: see Figure 3.1) strongly suggested that the intermediate forms of *Calliandra* found at Flores, Guatemala were of hybrid origin with all the characters tested being intermediate between the two parents, *C. calothyrsus* and *C. houstoniana*. There was a high level of RAPD marker diversity for the intermediate taxon (Shannon's index S = 12.6) compared to the parent species (for both *C. calothyrsus* and *C. houstoniana*, S = 4.5). Diagnostic RAPD markers for *C. calothyrsus* and *C. houstoniana* were revealed but were not, however, additive in the intermediate form. Hence, conclusive evidence for hybridity from RAPD markers was not available, although the high value of Shannon's index exhibited by the intermediate relative to its putative parents was itself evidence of hybrid origin.

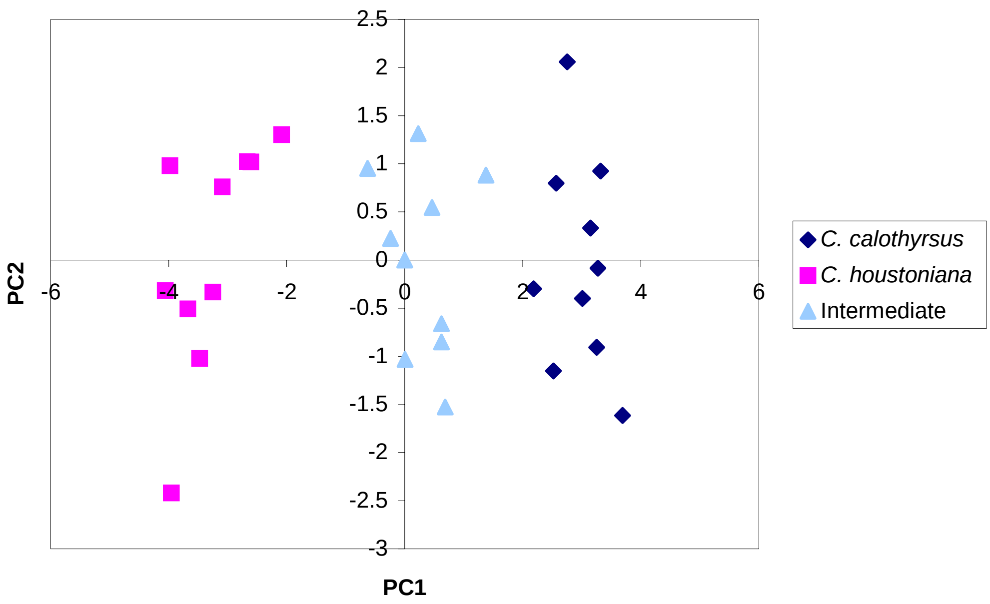

Figure 3.1: PCA plot of the first two principal components for morphometric traits of *C. calothyrsus*, *C. houstoniana* subsp. *houstoniana* and the putative hybrid (Intermediate) collected from Flores, Guatemala.

3.2. Artificial hybridisation

3.2.1. Background and methods

Controlled pollinations were carried out between three species of *Calliandra* planted in 1992 in a trial at La Colonia, Siguatepeque, Honduras by CONSEFORH to determine the cross-compatibility within *Calliandra* series *Racemosae*. Reciprocal crosses were made between flowers of *C. calothyrsus, C. houstoniana*, and *C. juzpeczukii*. Seed resulting from the above experiment was planted as a trial at Lancetilla Botanic Gardens, Tela, Honduras to monitor the growth and reproductive success of the hybrids.

3.2.2. Results

All inter-specific cross pollinations were successful with the exception of those between *C. calothyrsus* and *C. juzpeczukii* (Table 3.1). The percentage fruit set was greatest in the intra-specific crosses, and generally lower in the inter-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 (4.43% as opposed to 18.63%), perhaps suggesting that *C. calothyrsus* has a stronger incompatibilty mechanism than *C. houstoniana*. *C. juzpeczukii* did not set any fruits as a result of selfing and fruit set was corresponding low for *C. houstoniana* after selfing.

Early results from the hybrid trial at Lancetilla Botanic Gardens indicated that the fastest growing hybrid, in terms of plant height, was that between *C. houstoniana* and *C. calothyrsus*. This hybrid was faster growing than either of its parents, and significantly taller than its reciprocal cross, *C. calothyrsus* x *C. houstoniana*. These results were obtained after only six months growth, hence any conclusions drawn from them should be tentative, but there does appear to be evidence of hybrid vigour, and the role of *C. houstoniana* as the maternal parent, both in terms of the growth of the hybrid and the species' incompatibility mechanism merits further investigation. The production of inflorescences was also monitored in the trial, and structurally perfect flowers were produced by *C. calothyrsus* and all the hybrids. Neither *C. houstoniana* or *C. juzpeczukii* had flowered at this stage, which was not unusual as they do flower at a later stage of maturity than *C. calothyrsus*. Precocious floral development does seem to be characteristic of their hybrids, however.

Table 3.1: Mean percentage fruit sets and seed ovule ratios for inter-specific crosses made between *C. calothyrsus*, *C. houstoniana* and *C. juzpeczukii* (standard errors in parentheses).

Species	Cross	Mean % fruit set	Mean seed ovule ratio
C. calothyrsus	Self	2.10 (1.08)	0.419 (0.04)
	C. calothyrsus x *C. calothyrsus*	22.24 (2.85)	0.726 (0.02)
	C. calothyrsus x *C. houstoniana*	4.43 (1.35)	0.314 (0.05)
	C. calothyrsus x *C. juzpeczukii*	0.00 (0.00)	0.00 (0.00)
	Open pollination	5.66 (1.76)	0.575 (0.01)
C. houstoniana	Self	0.58 (0.44)	0.201(0.03)
	C. houstoniana x *C. calothyrsus*	18.63 (7.87)	0.369 (0.03)
	C. houstoniana x *C. houstoniana*	24.00 (2.45)	0.601 (0.12)
	C. houstoniana x *C. juzepczukii*	22.20 (2.25)	0.618 (0.09)
	Open pollination	6.31 (2.84)	0.590 (0.05)
C. juzepczukii	Self	0.00 (0.00)	0.00 (0.00)
	C. juzpeczukii x *C. calothyrsus*	0.00 (0.00)	0.00 (0.00)
	C. juzepczukii x *C. houstoniana*	21.33 (6.73)	0.749 (0.02)
	C. juzepczukii x *C. juzepczukii*	38.83 (9.95)	0.737 (0.05)
	Open pollination	9.44 (3.47)	0.433 (0.14)

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

Natural hybridisation

- Although sympatric populations of *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*. The hybrid appeared to show hybrid vigour and reduced fertility.

Implications: With the increasing disturbance of natural forest habitats, the incidence of inter-specific hybridisation in *Calliandra* could be expected to 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 any other *Calliandra* species. Seed should not be collected from stands where more than one species are intimately mixed. *Ex-situ* conservation stands and seed production orchards should be excluded from other sources of *Calliandra* pollen.

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 in terms of height of the main stem.
- Precocious flowering may be associated with the 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, there may be hybrid vigour for growth characteristics, and crosses between *C. houstoniana* and *C. calothyrsus*, with *C. calothyrsus* as the pollen parent, may be the most productive. The fertility of such a hybrid is open to question given the conflicting observations between natural and artificially produced hybrids.

For further information, refer to:

CHAMBERLAIN, J.R. and HUBERT, J.D. (2001). Reproductive biology and seed production. pp. 13-28. In: *Calliandra calothyrsus*, an agroforestry tree for the humid tropics. (Ed.) Chamberlain, J.R. Tropical Forestry Paper 40, Oxford Forestry Institute, Oxford, UK.

CHAMBERLAIN, J.R. and HUBERT, J.D. (2001). Molecular analysis of genetic variation. pp. 67-76. In: *Calliandra calothyrsus*, an agroforestry tree for the humid tropics. (Ed.) Chamberlain, J.R. Tropical Forestry Paper 40, Oxford Forestry Institute, Oxford, UK.

CHAMBERLAIN, J.R., MACQUEEN, D.J., HUBERT, J.D. and DUNSDON, A.J. Patterns of variation in *Calliandra calothyrsus* Meisn. (Mimosoideae: Leguminosae). Under revision for *Plant Systematics and Evolution*.

4. Important resource populations that are under threat, or of low genetic diversity, will be identified for conservation. Combined with the results from the breeding system and hybridisation experiments, a rational conservation strategy for the species will be developed.

Status: *Achieved*

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 and reproductive biology.

4.1. Background and methods

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 1996; 1998; 2001), and were used to help prioritise populations of *C. calothyrsus* for conservation using three methodological approaches.

The most widely accepted method of measuring 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). 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.

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.

Thirdly, 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.

4.2. Results

For *C. calothyrsus*, IUCN ratings were assigned to the species as a whole, and to the four subgroups within it (Table 4.1). *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. Using the Star rating 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 4.1). The strategy employed by Graudal *et al*. (1997) was also used to identify specific populations of *C. calothyrsus* for conservation (Table 4.2).

Table 4.1: Conservation status of *Calliandra calothyrsus* and its subgroups.

Species/subgroup	IUCN Threat category[3]	Star Rating[4]	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).

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*. C*irca 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 (recommended under the review and implementation of the future of the OFI seed collections; Chamberlain, 1999). 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 4.2 should also be given high priority to facilitate the supply of productive seed, whilst ensuring the conservation of important resource populations.

[3] 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.
[4] Star Rating based on Hawthorne (1996): Gold = moderate conservation concern; Blue = minor conservation concern; Green = no conservation concern.

Table 4.2: 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; c*irca 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; c*irca 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 c*irca 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; c*irca 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*.

Summary
Conservation status of *Calliandra 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.

For further information, refer to:

CHAMBERLAIN, J.R. (1998). Isozyme variation in *Calliandra calothyrsus*: its implications for species delimitation and conservation. *American Journal of Botany* **85** (1): 37-47.

CHAMBERLAIN, J.R. (2001). Conservation. pp. 77-85. In: *Calliandra calothyrsus*, an agroforestry tree for the humid tropics (Ed.) Chamberlain, J.R. Tropical Forestry Paper 40, Oxford Forestry Institute, Oxford, UK.

CHAMBERLAIN, J.R. and HUBERT, J.D. (2001). Molecular analysis of genetic variation. pp. 67-76. In: *Calliandra calothyrsus*, an agroforestry tree for the humid tropics. (Ed.) Chamberlain, J.R. Tropical Forestry Paper 40, Oxford Forestry Institute, Oxford, UK.

5. A synthesis of the state of knowledge surrounding *C. calothyrsus* published in the form of a Tropical Forestry Paper (TFP).

Status: *Achieved*

A monograph on the current state of knowledge surrounding *C. calothyrsus* was developed to provide a summary of the research results of this project. The monograph also provides information on the taxonomy, biology, provenance evaluation and genetic diversity of the species (largely based on the results of FRP projects R4485, R5728 and R6551), as well as providing a wealth of information on the practical use of the species. In addition, the monograph 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.

It is hoped that the monograph, published with OFI’s Tropical Forestry Paper series, 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.

For further information, refer to:

CHAMBERLAIN, J.R. (Ed.) (2001). *Calliandra calothyrsus*: an agroforestry tree for the humid tropics. OFI Tropical Forestry Paper No. 40. Oxford Forestry Institute, Oxford, UK. 101 pp.

6. The potential for pollen contamination between seed orchards of *C. calothyrsus* determined.

Status: *Achieved*

6.1. Background and methods

Three seed orchards of the important multi-purpose tree, *Calliandra calothyrsus*, were established at the KARI research station at Embu, Kenya in collaboration with ICRAF. *C. calothyrsus* is an important tree to the area and is cultivated extensively as a source of fodder for dairy cattle (NARP, 1998). Farmers in the area have found the provision of *C. calothyrsus* fodder in the diets of cattle to improve milk yield and quality, and reduce the costs associated with the provision of commercial feed supplements. Because of the economic importance of *C. calothyrsus* to the area, the early results of OFI's international *Calliandra* provenance trials were used to identify seed sources of the species with high biomass yields. The local landrace (Embu) and two provenances from the species native range (Patulul, a provenance that is genetically similar to the Embu material, and San Ramón) were selected for the establishment of seed orchards in collaboration with FRP project R6551 to supply seed/planting material to meet future demand for *C. calothyrsus* in Embu and elsewhere.

The existence of these orchards provided an ideal opportunity to test the recommended seed orchard isolation distance suggested by the results of this project (Chamberlain, 2000; 2001). Pollen contamination of seed orchards could result in the loss of provenance identity of the seed produced in the orchards. This could have unpredictable results for farmers planting *C. calothyrsus* as the San Ramón provenance is very different in form and morphology from the two other provenances. The maintenance of a genetically variable but stable source of germplasm is critical to the farmers who use *C. calothyrsus* in the Embu region, and for the reputation of ICRAF, KARI and KEFRI as suppliers of reliable, well-documented germplasm. Isozyme markers were proposed for the study of pollen flow between the three seed orchards.

Each of the three seed orchards was established with seed from a single provenance. The Ex. San Ramón orchard was established approximately two kilometres from the Ex. Patulul and Embu orchards. The latter two orchards, which are similar in terms of morphology and genetic variation, are separated by a distance of less than 500 m. Seed (kept separate by tree and month during 1998) from up to 20 trees in each of three seed orchards of *C. calothyrsus* (Embu, Ex. Patulul and Ex. San Ramón) were sampled for isozyme analysis (Table 6.1; Figure 6.1). Ten seeds per tree were randomly sampled for examination.

Isozyme electrophoresis and analysis was carried out according to Chamberlain (1998). Two enzyme systems were used to resolve isozymes: alcohol dehydrogenase (ADH) and malate dehydrogenase (MDH). A genotype was assigned to each seed sampled, and the allele frequencies at each locus in each population were calculated using BIOSYS-1 (Table 6.2; Swofford, 1989).

Table 6.1: Number of trees sampled for isozyme analysis from each of three seed orchards of *Calliandra calothyrsus* over a period of four months.

Seed source	Number of trees sampled			
	June	**July**	**August**	**September**
Embu	5	5	20	17
Ex. Patulul	5	5	11	12
Ex. San Ramón	3	3	8	10

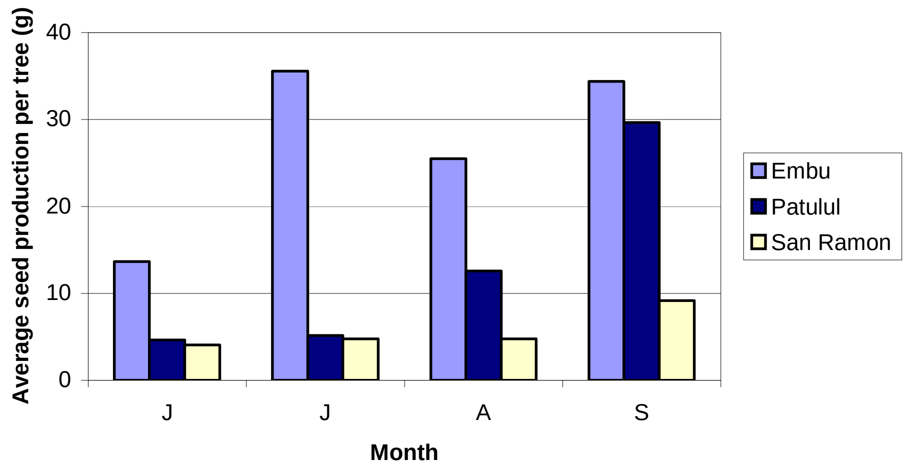

Figure 6.1: Seed production per tree in 1998 from three seed orchards of *Calliandra calothyrsus* planted at Embu, Kenya.

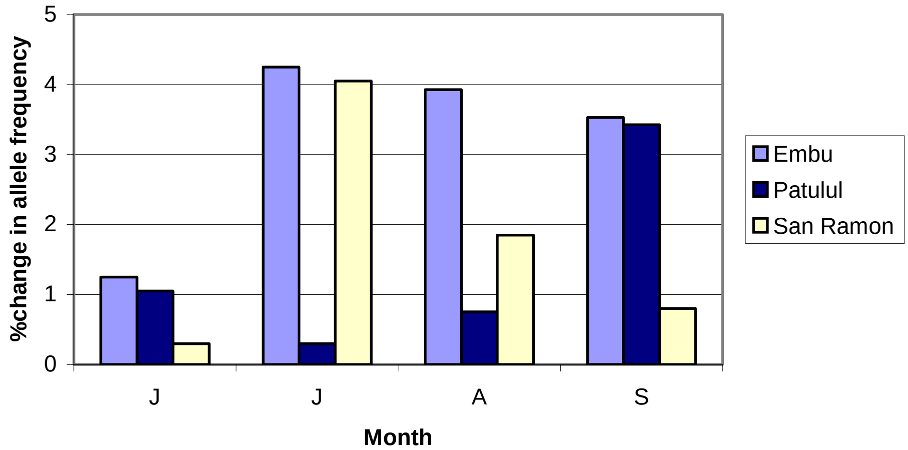

Figure 6.2: The percentage change in allele frequencies in the seed progeny of three seed orchards of *Calliandra calothyrsus* when compared to those in the original population.

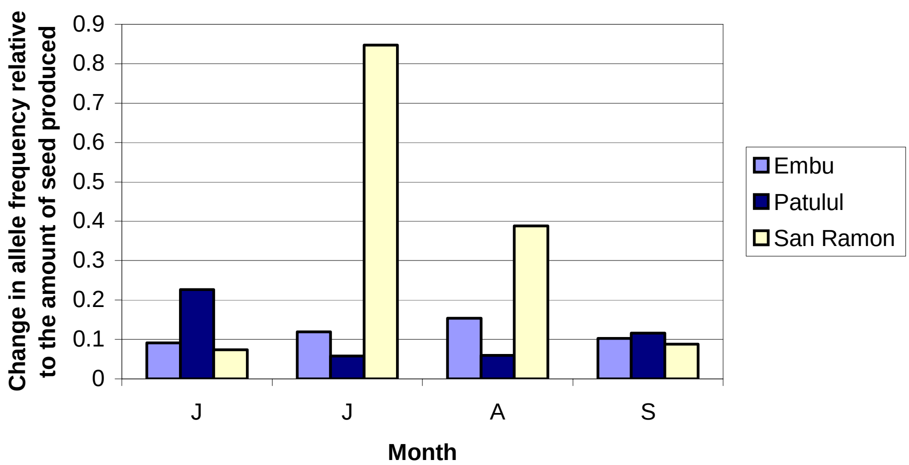

Figure 6.3: Change in allele frequencies as a proportion of the average amount of seed produced per tree in three seed orchards of *Calliandra calothyrsus*.

Table 6.2: Summary of allele frequencies for seven isozyme loci among all seed sources sampled.

Locus	Allele	Orchard														
		Embu					Ex. Patulul					Ex. San Ramón				
		Original population[5]	Jun	Jul	Aug	Sept	Original population	Jun	Jul	Aug	Sept	Original population	Jun	Jul	Aug	Sept
Adh-1	a	1.000	0.990	0.980	0.942	0.903	1.000	0.970	1.000	0.991	0.867	1.000	1.000	1.000	1.000	1.000
	b	0.000	0.010	0.020	0.058	0.097	0.000	0.030	0.000	0.009	0.133	0.000	0.000	0.000	0.000	0.000
Adh-2	a	0.000	0.010	0.130	0.072	0.029	0.012	0.000	0.000	0.000	0.008	0.988	1.000	1.000	1.000	0.995
	b	1.000	0.990	0.870	0.928	0.971	0.988	1.000	1.000	1.000	0.992	0.012	0.000	0.000	0.000	0.005
Mdh-1	a	0.000	0.030	0.020	0.027	0.015	0.000	0.000	0.000	0.009	0.000	1.000	1.000	1.000	1.000	1.000
	b	1.000	0.970	0.980	0.973	0.985	1.000	1.000	1.000	0.991	1.000	0.000	0.000	0.000	0.000	0.000
Mdh-2	a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000
	b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000
Mdh-3	a	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	0.850	0.938	0.975
	b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.150	0.062	0.025
Mdh-4	a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000
	b	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000
Mdh-5	a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000	0.000	0.000
	b	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000	1.000	1.000

[5] From Chamberlain (1998)

6.2. Results

Seed production and the role of pollinators: Seed production data was recorded in all three orchards during 1998 (Figure 6.1). The Embu orchard was the most productive, which may relate to the older age of the trees when compared to Ex. Patulul and Ex. San Ramón, and the adaptation of the orchard to the site and its pollinators. Seed production per tree was generally greater in September than in any other month, although for the Embu orchard seed production per tree was greatest in July.

Preliminary observations on potential pollinators visiting the three orchards during July 1999, suggested that bats visited infrequently during this period, but birds were abundant visitors to the Embu orchard early in the morning. At sunrise, the receptivity of the floral stigmas has reduced, but the large number of birds visiting the orchard during this period may allow successful pollination and fertilisation of ovules. Further observations of the potential pollinators at this site is required to determine the role of bats versus birds in pollinating *C. calothyrsus* at this site.

6.2.2. Estimates of pollen contamination between orchards: If the allele frequencies at the loci Adh-1, Adh-2, Mdh-1 and Mdh-3 (i.e. those loci showing variability) for the seed progeny of the trees sampled are compared to the allele frequencies in the original population (from Chamberlain, 1998), the percentage change in frequency can give some estimation as to the potential for pollen contamination between the Ex. Patulul and Embu orchards and the Ex. San Ramón orchard. Overall, the percentage change in allele frequency is low (range 0.30-4.25%; mean 2.12%), but variable between seed orchards and month (Figure 6.2).

If the change in allele frequencies relative to the amount of seed produced (which perhaps gives a more significant estimate of the potential for pollen contamination) is considered, the proportion resulting is greatest for the Ex. San Ramón progeny during July and August than for any other orchard during any other month (Figure 6.3). This might suggest that pollen flow from Embu and Ex. Patulul, had the greatest effect on the resultant Ex. San Ramón progeny during the flowering period corresponding to the production of this seed.

There is no data on the production of hermaphrodite and staminate flowers available for the period prior to the collection of the seed in 1998. However, in general the higher the number of hermaphrodite flowers produced, the greater will be the quantity of seed produced at the end of the corresponding fruiting period. Hence, greater numbers of flowers in one orchard of *C. calothyrsus* at Embu, might result in the increased potential for pollen flow over a larger area, and an increase in the potential for pollen contamination in orchards with low levels of flowering during the same period.

Summary
The potential for pollen contamination between seed orchards of *C. calothyrsus*

- The potential for pollen contamination between the three seed orchards of *C. calothyrsus* planted at Embu is low.
- Differences between orchards in flowering time and the quantity of flowers produced may increase the potential for pollen contamination.
- Studies on the pollinators of *C. calothyrsus* at this site were inconclusive and require further study.

Implications: The seed orchard isolation distances recommended by this project are sufficient to maintain a low level of pollen contamination. However, differences between orchards in flowering time and the quantity of flowers produced may increase this potential.

Contribution of Outputs

As a continuation of FRP Project R5728, the results of the project provide a firm foundation for the future improvement and use of *C. calothyrsus*. The species is used to provide a number of products, e.g. fuelwood, fodder, green manure and honey, and services such as land rehabilitation, erosion control and shade. *C. calothyrsus* has particular potential for acidic soils on which many of the other popular agroforestry trees perform poorly. Until recently, little work had been done to evaluate or improve the current planting stock, or address problems experienced when propagating the species. Both R5728 and R6535 aimed to address these problems by making seed material of superior provenances available to researchers and ultimately farmers. A greater understanding of the reproductive biology, breeding system and pollination system of *C. calothyrsus* has facilitated a more pragmatic approaches to genetic improvement, the design of seed orchards and improving seed production. Opportunities also exist for evaluating species hybrids of *C. calothyrsus* and conservation of valuable or endangered populations, both *in situ* and *ex situ*.

These problems are directly related to the FRP Forest/Agriculture Interface Production System and will contribute to increased productivity of tree/crop based systems involving *C. calothyrsus*.

The importance of this project is highlighted by the great interest shown throughout the humid tropics in improved material of *C. calothyrsus,* particularly on sites with acid soils. Orders for seed continually outstrip possible supply from the native range. There is considerable momentum in terms of scientific research, international collaboration, and world-wide interest in the results of R6535. The perception of the species usefulness is very high with FACTNET (Forest, Farm and Community Tree Network, formerly NFTA) ranking studies on *C. calothyrsus* and species for acid soils first in an international survey. Through active follow up it is hoped that the users of the species will not only have access to well documented material of superior provenances, but will be able to use it in ongoing improvement programmes. The results of this project will give them the information they need.

Follow-on Activities

Seed Production Field Manual

Investigations into the reproductive biology of *Calliandra calothyrsus*, and seed production initiatives set up by the author in Central America, by CSIRO in Australia and by ICRAF/KARI/KEFRI in Kenya have given rise to a wealth of information on seed production and how it is controlled. The publication of a field manual on 'Improving Seed Production in *Calliandra calothyrsus*' was proposed in 1998 as an additional output to this project, and provides a synopsis of the current state of knowledge with regard to *C. calothyrsus* seed production and how to improve it. The need for, and potential value of such a manual was recognised by a variety of researchers and field workers including those within international research institutes (ICRAF, CIFOR, CSIRO), and NGO's, e.g., FACTNET, BAIF (India) and FSP (Laos). These organisations have documented their own experiences with producing seed of *C. calothyrsus* during verbal and written communications in the last two years. Constraints to the production of seed, and possible means by which they may be overcome have been discussed and these views incorporated into the seed production manual. Generally it has been agreed that such a manual would be of great value and allow other researchers to begin seed production in *C. calothyrsus* from a much better position than has been previously possible (see below).

Reviewers comments on
'Improving Seed Production in *Calliandra calothyrsus*: A field manual for Researchers and Extension Workers'

'*The manual will be very useful to some of the NGOs we work with here in Southeast Asia*.' - Jim Roshetko, Research Scientist, Winrock International (seconded to ICRAF), Indonesia.

'*The seed production manual on calliandra is highly informative and easy-to-understand*.' - Dr Joshua Daniel, Senior Research Scientist, BAIF, India.

'*I like the text and the illustrations very much. You've explained some complex concepts very clearly, and it will make an excellent contribution to the extension material available on C. calothyrsus*.' - Dr Ian Dawson, Germplasm Specialist, ICRAF, Kenya.

'*I thoroughly enjoyed reading the text, and learnt some new information myself, particularly about the pollination of calliandra. The text will be of great assistance to the extension services that we work with; it is clear, nicely laid out, with one concept to a page or facing pages, which makes use and dissemination of information very easy. The whole section on seed production on farm is very well explained, very useful and answers directly the questions that farmers have in establishing seed production on farm. The emphasis that seed production can be incorporated into the existing niches and uses of trees is often neglected, with extension workers and farmers perceiving that specific seed lots have to be set up specifically for seed production*.' - Christine Holding Anyonge, Agroforestry Extension Advisor, Regional Land Management Unit, Sida, Kenya.

'*The calliandra field manual is an excellent, informative, clearly laid-out and illustrated guide which is very simple to use. Can we have some copies as soon as it is published?*' - Gabriel Pol, Director, Evergreen Trust, Tanzania.

'*This manual will definitely come in handy for people in the field. Please send me copies in English and Spanish so that I can send them to our field staff*.' - Stuart Conway, Director, Trees, Water and People, Central America.

'*A very nice job!*' - John Weber, Forest Geneticist, ICRAF, Peru.

The manual is aimed at researchers wishing to set up seed production areas of *C. calothyrsus*, and at NGOs and fieldworkers who wish to produce seed at the farm level. The manual is illustrated with diagrams and colour plates, and has been produced in four different versions; two in English aimed at African and Asian audiences, one in Spanish aimed at Latin Americans, and a fourth in Indonesian produced in collaboration with ICRAF-Bogor. The manual has been distributed to over 200 individuals or organisations and was used as the focus for training workshops implemented in Kenya and Indonesia in November 2000 (Chamberlain, 2001; Chamberlain and Stewart, 2001). Approximately 60 extra copies of the manual were deposited with ICRAF in Nairobi and Bogor for later distribution, the manual has been advertised in the IUFRO Seed Physiology and Technology Newsletter and LeucNet News, and is available for downloading on the CNRD website in pdf form.

Seed Production Training Workshops

Two training workshops were implemented in collaboration with ICRAF in Nairobi, Kenya and Bogor, Indonesia in November 2000. The aim of the training workshops was to provide training on the reproductive biology of *C. calothyrsus* and assess the implications for improving seed production in the species. The workshop in Indonesia was implemented in collaboration with Ms Janet Stewart (FRP Project R6549) who gave training on the utilisation of *C. calothyrsus* for fodder. This workshop was organised in collaboration with Mr Jim Roshetko, who is employed by Winrock International but seconded to ICRAF in Bogor, Indonesia. The workshop in Kenya was organised in collaboration with Programme 2 (Tree Domestication) of ICRAF. The workshop was held at ICRAF headquarters in Nairobi, Kenya and was combined with a field visit to Embu, organised in collaboration with the Kenya Agricultural Research Institute (KARI) and the Kenya Forestry Research Institute (KEFRI).

Both workshops ran over a period of three days and involved a mixture of presentations, group discussions and a field visit. Knowledge of *C. calothyrsus* and its uses differed between the two workshops. In Indonesia, there was considerable awareness surrounding the use of calliandra for a variety of purposes, however, much of the information discussed (e.g., fodder use, seed production, seed processing and storage) was new to a large majority of the participants. *C. calothyrsus* seemed to be most well-known as a means of preventing soil erosion on sloping land, especially in the east of Indonesia, or from forest land where the species has been planted as part of ‘re-greening’ programmes. Only a few of the participants were engaged in seed collection or production.

In Kenya, all participants were familiar with the use of calliandra, particularly as a source of fodder for livestock. Many of the participants were engaged in, or planning to start, calliandra seed production activities. For example, participants were engaged in the on-station establishment of seed orchards (with high biomass producing provenances of calliandra), were working with farmer groups engaged in seed collection, and developing on-farm nurseries and seed production.

Both workshops were implemented successfully and the majority of the participant’s expectations for the workshop were achieved. All participants developed a personal plan of action, which stated how they hoped to use the information and resources they obtained at the workshops in their own work. Additional outputs from the workshops include the translation of the field manual ‘Improving seed production in *Calliandra calothyrsus*’ into Indonesian by ICRAF/Winrock. It has been suggested that a short section on seed processing and on-farm storage is also included in this version, and the list of seed suppliers reflects additional local sources. Permission has also been granted by Winrock International to translate their field manual on *C. calothyrsus* (1997) into Indonesian. This will include an update of the section on fodder and seed production.

For further information, refer to:

CHAMBERLAIN, J.R. (2000). Improving seed production in *Calliandra calothyrsus*: a field manual for researchers and extension workers. Miscellaneous Paper, Oxford Forestry Institute, Oxford, UK. 37 pp. English, Indonesian and Spanish.

CHAMBERLAIN, J.R and STEWART, J.L. (2001). Calliandra Seed Production and Utilisation. Report on a Training Workshop, November 14-16, 2000, Bogor, Indonesia. Projects R6535 and R6549. Oxford: Centre for Natural Resources and Development. pp. 18.

CHAMBERLAIN, J.R (2001). Calliandra Seed Production. Report on a Training Workshop, November 28-30, 2000, Nairobi, Kenya. Project R6535. Oxford: Centre for Natural Resources and Development. pp. 12.

Promotion Pathways

Dissemination outputs:

The project outputs will be promoted principally through the following dissemination outputs:

1. 'Improving seed production in *Calliandra calothyrsus*: a field manual for researchers and extension workers', Miscellaneous Paper, Oxford Forestry Institute, Oxford, UK. English, Spanish and Indonesian.
2. '*Calliandra calothyrsus*: an agroforestry tree for the humid tropics', OFI Tropical Forestry Paper No. 40. Oxford Forestry Institute, Oxford, UK. English.
3. Refereed scientific papers.
4. The implementation of project training workshops with follow-up work by participants.

Links with other organisations and projects:

R6535 has had close links with FRP Projects R6551 (Evaluation of selected non-industrial tree species and development of approaches to facilitate the utilisation of results) and R6549 (Fodder quality in *Calliandra calothyrsus*). Seed production has either been a focus these project's research activities, or recognised as a constraint to future work. The collaborators (BAIF, CIAT, FSP, ICRAF, KARI and KEFRI amongst others) of both R6551 and R6549 have been recipients of both the TFP and field manual on *C. calothyrsus*, and through their research and development work, are likely to be important to the uptake pathways for the results of R6535.

R6535 has also had close links with ICRAF in Kenya and Indonesia through direct collaboration. ICRAF-Kenya has plans to continue their research on seed production in *C. calothyrsus* through the continued monitoring of seed orchards in Embu, and new on-farm seed production research in the peri-urban areas of Nairobi, Kiambu, Embu and Meru. ICRAF-Indonesia is implementing a Forest Seed Project (IFSP) with funds from the Danish government, and plans to use the results of R6535 in this work. IFSP has funded the translation and publication of 'Improving seed production in *Calliandra calothyrsus*: a field manual for researchers and extension workers'.

Project Publications

Journals. Books, Manuals and Conference Papers

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