RURAL INDUSTRIES RESEARCH
& DEVELOPMENT CORPORATION

Potential for Seed Gum Production from *Cassia brewsteri*

A report for the Rural Industries Research and Development Corporation

by *David Cunningham, Kerry Walsh and Eric Anderson*

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Foreword

Seed galactomannans are widely used for a variety of industrial applications. The Australian market is supplied entirely by imports costing tens of millions of dollars per year. Carob, guar, and Senna gums are currently used to supply the bulk of this demand. However, inconsistency of supply and price has driven industrial users to search for alternative sources of supply.

Cassia brewsteri is a native tree of Central Queensland with galactomannans in its seeds. To assess the potential commercial use of this source, information was required on the gum quality, gum yield from seed, seed yield per hectare, and costs of production as well as the biology of the species.

This study has found commercial exploitation of this native legume species for gum production is not recommended at this time. However, the study has yielded useful data for the management of this species, in terms of its genetic diversity, distribution and habitat, phenology, propagation, and insect pests.

This project was funded from industry revenue which was matched by funds provided by the Federal Government.

This report, a new addition to RIRDC's diverse range of over 600 research publications, forms part of our New Plant Products R&D program, which aims to facilitate the development of new industries based on plants or plant products that have commercial potential for Australia.

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Peter Core
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This document is a condensed version of David Cunningham's (2000) PhD thesis on the 'Autecology of *Cassia brewsteri* with respect to galactomannan production.' Central Queensland University, Rockhampton.

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Executive summary

Cassia brewsteri (*Caesalpiniaceae*) is a tree endemic to central Queensland. In this project, the potential of the plant as a source of seed gums (galactomannans) with industrial applications has been assessed in terms of genetic diversity, ecological requirements, phenology, propagation, insect pests, seed chemistry, gelling characteristics and economic viability. Randomly Amplified DNA Fingerprints were used to indicate the level of genetic variation within the Australian *Cassia.* Phenetic analyses supported the maintenance of the four native *Cassia* taxa at species level and the division of *C. brewsteri* into two subspecies. Ecological parameters associated with 124 sites of natural or cultivated occurrence were characterised in terms of soil and vegetation type, and potential cultivation areas predicted using the climate modelling software BIOCLIM. A broad area of eastern Queensland was shown to be suitable for the cultivation of the tree. Flowering and fruiting phenology was documented and found to allow for a confined harvest period (once per year). Seed germination was achieved most effectively by mechanical scarification while propagation by rooted cuttings was unsuccessful. Heavy predation of seed by *Caryedon serratus* (an exotic bruchid) was noted. The potential impact of this insect on the ecology of *Cassia brewsteri*, and the potential for infestation of *Arachis hypogaea* (peanut) is discussed. *Cassia brewsteri* seed galactomannan was demonstrated to be acceptable for use as a food gum. It is comparable to *Ceratonia siliqua* (carob) gum in gelling strength, and contains less than 10 ppm of the toxin chrysophanic acid.

Based on the yield data obtained in the above mentioned studies, preliminary estimates were made of return on investment from plantation culture of *Cassia brewsteri* for seed production. This analysis suggests that production is not economic at current yields and seed gum prices. However, production of this commodity has potential, and reconsideration at a future time is recommended. A pilot planting, perhaps installed as part of a revegetation exercise (e.g. minesite rehabilitation), would be a useful resource from which to base future assessments.

Introduction

Cassia brewsteri

Cassia brewsteri (F.Muell.) F.Muell. ex Benth. (Caesalpiniaceae) is a small to medium tree endemic to Queensland, Australia. It has previously been identified as a species with potential as a multipurpose tree in agroforestry with possible utilisation as a source of sawn timber, fuelwood, fodder, or pharmaceutical products.

C. brewsteri wood is moderately dense (850 kg/m^3), with potential as fuelwood or a useful general-purpose hardwood and cabinet timber. The wood is pale pink with yellowish sapwood, open grain and no figure (Turnbull *et al.* 1986). The tree has the ability to coppice (Ryan and Bell 1989) and is not susceptible to termite attack in the field (Mitchell 1989).

Vercoe (1987, 1989) investigated the fodder value of *C. brewsteri* and 38 other Australian tree species. Dry Matter Digestibility (DMD) was estimated *in vitro* from chemical composition (e.g. crude protein level of 11.9%) by comparison with standard samples of species with known *in vivo* digestibility. The predicted *in vivo* DMD of 61.6% of *C. brewsteri* foliage was suggested to be adequate to provide subsistence forage. However, Vercoe noted that neither the foliage nor the twigs by themselves are adequate for the maintenance of sheep or cattle. The foliage is deficient in P and K whilst the twigs are deficient in crude protein, P, K and Na. Although *C. brewsteri* was recommended for further study no other research has been published on the feed potential of the tree.

Further, while digestible *in vitro*, the foliage and fruit are unpalatable to cattle. There is therefore no potential to develop the plant as a fodder crop in Australia. Indeed, as the tree produces root suckers after clearing, graziers consider it a weed in parts of central Queensland (Kleinschmidt and Johnson 1977, Scanlan and Fossett 1981). Agricultural studies of *C. brewsteri* have focussed on means of culling the plant (e.g. Back 1974).

The tree is useful as a shade tree and is reported to tolerate saline and alkaline soils (Doran *et al.* 1997). The floral blooms and deep green foliage make it an attractive ornamental species grown in Queensland, New South Wales and even Victoria under sheltered conditions (e.g. Francis 1981, Elliot and Jones 1982).

Positive alkaloid and negative anti-tumour tests of bark tissue extracts of *C. brewsteri* have been reported (Collins *et al.* 1990). No other pharmaceutical applications of *C. brewsteri* have been published.

Seed gums

Vegetable gums are becoming increasingly common in foods as they play important roles in both the manufacturing process and the mouthfeel or texture of the products. At present there are 38 additives classed as "thickener/vegetable gum" which have been approved for use in Australian food (ANZFA 1999). Sixteen of these are starches in various forms, nine are seaweed extracts, three are synthesised by bacteria and three are exudates from the branches of trees. Two of the vegetable gums are sourced from seeds and are known as seed gums or galactomannans.

Galactomannans are polysaccharides stored in the seeds of many plants of the legume family. These polymers of mannose and galactose are the functional components of seed gums. Carob (410) and guar (412) are the only seed gums currently approved for use in Australian food. Tara (417) and *Senna tora*/*obtusifolia* ('cassia') gum (no international additive code) are other seed gums which are used in foods overseas. *S. tora/obtusifolia* gum is used internationally and in Australia as a source of vegetable gums mainly for the pet food and textile industries. The product is currently exclusively made by Diamalt, and is marketed as 'cassia gum' or Diagum™ CS. It is made from the processed seed of both *S. tora* and *S. obtusifolia* collected from wild populations of these plants in India.

Seed gums are used in a wide range of food products and processes to thicken solutions, form an aqueous gel with other polysaccharides and prevent syneresis (the separation of liquid from a gel that is caused by contraction). The concentration of seed gum in foods varies, typical concentrations of galactomannans at 0.5-1.0% can achieve the same viscosity as starches at 4.0-6.0% (Fox 1997). Seed gums are commonly used in conjunction with carrageenan (407) from seaweed, or xanthan gum (415) from bacteria. The interaction between the seed gum and the other polysaccharide results in a stronger, more elastic gel, or more viscosity, than that produced using either gum by itself.

Some of the many foods containing galactomannans are ice cream, other milk-based products and desserts, mayonnaises, dressings, sauces and deep-frozen foods. Non-food uses of galactomannans include lubrication of oil drills, waterproofing of underwater explosives and as a flocculant in paper and textiles manufacturing (Dea and Morrison 1975, Coppen 1995).

One feature of galactomannans is that although they are comprised of simple sugars they are not digested by human enzymes. They are commonly used in diet or 'lite' foods since they add texture and a creamy mouthfeel to foods but pass through the body undigested. Guar has even been marketed as a diet tablet which swells in the stomach to alleviate the sensation of hunger. While this use may be of dubious health value, galactomannans do have some dietary value as soluble fibre (Lössl 1989). For diabetics, galactomannans also have the benefit of slowing the passage of food in the stomach thereby slowing the resorption of sugars such as glucose.

Seed galactomannans are competitively priced in comparison to other vegetable gums. They are unmodified natural products with some health benefits and are widely accepted by consumers. It is likely that their use in foods will continue to increase and that new food uses and products will be developed.

Many plants have been chemically analysed for their potential as a source of seed gums, results for over 120 species have been reported in the public domain. The two newest seed gums on the world market have not yet developed to the stage of crop production, but rather are currently harvested from wild *Senna* (in India) and tara (in Peru). *Senna tora/obtusifolia* gum is approved for food use in Europe, Japan and the USA (Hallagan *et al.* 1997). Tara is approved for food use in Europe (Borzelleca *et al.* 1993). At present, *Senna* gum is used only in petfoods in Australia while tara is not known to be used in any applications in Australia.

The world market for vegetable gums used in foods was estimated at US$10 billion in 1993 (Coppen 1995), the majority being the seaweed gums and the starches. Virtually all of Australia's requirements for vegetable gums are met by imports costing an estimated AU$40 million a year, carob gum imports alone cost some AU$10 million a year Australia-wide (CRCIPB 1996). The volume and value of guar and *S. tora/obtusifolia* gums imported into Australia is uncertain since many types of industrial gum are grouped under the same import code used for statistical records.

For decades Australian researchers have investigated the potential to produce seed gums locally. Of these gums, carob gum has the longest history of use in industrial processes. In fact, people have used the carob tree in various ways for around 4000 years. The whole pods are a nutritious stockfeed and the dried pulp (carob powder) is used as a flavouring, for example as a substitute for chocolate. A native of the Mediterranean, the carob tree is suited to many parts of Australia and was first planted here in the 1890s. The first experimental orchards were established in the 1970s in South Australia but as yet there has been no local harvests of carob seed for gum production. Guar (*Cyamopsis tetragonolobus*) has also been grown on a small scale in Australia for decades without developing as a commercial seed gum crop. Renewed interest in local sourcing has led to further trials of guar in Queensland with a view to local production and processing of seed gums. The feasibility of *Senna tora* gum production in Australia is the subject of a current RIRDC project.

Other RIRDC projects relating to seed galactomannan production in Australia include:

- Commercial viability of *Senna tora* gum production in Australia (NPP00-65)
- Carob agroforestry in the low rainfall Murray valley (UCS-14A)

Mesquite (*Prosopsis* species) is another potential source of seed gums. Research in Brazil has went as far as pilot-scale processing of the seed for gum production but as yet there has been no mesquite gum traded on the world market. Mesquite was introduced to Australia in the early 1900s and has since become a serious woody weed, a factor which would probably impede its commercialisation here and in many parts of the world.

To assess the potential of *Cassia brewsteri* as a new seed gum crop information is required on a diverse range of factors. The aims of this project were to characterise *C. brewsteri* in terms of genetic diversity, distribution and habitat, reproductive phenology and the effects of an insect pest. The seed chemistry was characterised in terms of the level of a toxin, chrysophanic acid, the content and composition of galactomannan and the gelling interactions of the galactomannan with carrageen. An analysis of the commercial viability of the tree as a seed galactomannan crop in Australia was based on the new information and on comparison to similar crops.

1. Genetic diversity in the Australian *Cassia*

Abstract

Genetic diversity in *Cassia brewsteri* and the taxonomy of the Australian *Cassia* was assessed using Randomly Amplified DNA Fingerprints (RAFs). Thirty accessions of *C. brewsteri* collected from throughout its natural distribution, three accessions of *C. tomentella* and a single accession of each of *C. queenslandica* and *C. marksiana* were analysed with three random decamer primers. These primers yielded a reproducible amplification profile of 265 scorable polymorphic fragments for the 35 accessions. These three plus an additional three primers produced a total of 393 polymorphic fragments in a subset of nine representative accessions. The molecular markers were used to calculate similarity coefficients between each pair of individuals and these were analysed by Multidimensional Scaling (MDS) and phenogram. The analyses support the maintenance of the four native taxa at species level and the division of *C. brewsteri* into two subspecies.

Introduction

Taxonomy of Australian *Cassia*

Cassia (Caesalpiniaceae) is a pantropic genus of about 30 species worldwide, mostly small trees or tall shrubs. The genus is divided into eight series (Irwin and Barneby 1982) only one of which, *Obolospermae*, is represented naturally in Australia. There are either two or four species native to Australia depending on the system used. All four taxa were initially described as varieties of *C. brewsteri* (F.Muell 1859) and later raised to species level. The other three taxa formally described as species are *C. tomentella* (Benth. 1864, Domin 1926); *C. marksiana* (F.M.Bailey 1897, Domin 1926) and *C. queenslandica* (F.M.Bailey 1891, C.T.White 1939). The four species were retained in the last major review of *Cassia* in Australia (Symon 1966) and in the regional flora treatment for southeast Queensland (Stanley and Ross 1983). The Flora of Australia Volume 12 treatment (Randell and Barlow 1998) retained *C. queenslandica* as a distinct species but reverted the other three species to varieties of *C. brewsteri*. The nomenclature of Symon (1966) is used in the current study.

C. brewsteri was named after a Scottish scientist, Sir David Brewster, while *C. marksiana* was named for Dr C.F. Marks, a Queensland physician and amateur plant collector (Rowell 1991). The etymology of *C. queenslandica* refers to its provenance and that of *C. tomentella* refers to its tomentose leaves and pods. *C. brewsteri* is commonly known as Leichhardt bean, bean tree, Brewster's cassia, cigar cassia or golden shower tree. Common names for *C. tomentella* are velvet bean tree and velvet cassia, while *C. marksiana* is known as brush cassia, Mark's cassia, cigar cassia or native Laburnum. No common names have been reported for *C. queenslandica*.

The phylogenetic relationships of the four native taxa are uncertain. Symon (1966) links *C. tomentella* with *C. marksiana* based on pod characteristics, and *C. brewsteri* with *C. queenslandica* (no rationale given). Symon considered pod morphology as the most reliable way to distinguish these species. His revision of Australian *Cassia* retained *C. tomentella* and *C. marksiana* as distinct species based on differences with *C. brewsteri* in pubescence (pod surface), leaflet shape and geographic distribution. The main diagnostic differences given between *C. brewsteri* and *C. tomentella* were that the leaflet blades in *brewsteri* are glabrous above and sparsely pubescent beneath, whilst in *C. tomentella* they are glabrous and shining above, and densely minutely pubescent below. The pods of *C. tomentella* are also cylindric and densely minutely pubescent whereas *C. brewsteri* pods are compressed or cylindric. Where the ranges of *C. brewsteri* and *C. tomentella* meet, the leaves of *C. tomentella* are longer and less densely pubescent, suggesting a possible introgression, however intermediate pods have not been observed (Symon 1966, Stanley and Ross 1983). The apparent intergradation between *C. brewsteri* and *C. tomentella* in geographical areas where they overlap was the basis for reverting to the varieties described in the Flora of Australia treatment (Randell and Barlow 1998, Randell pers. comm.).

C. brewsteri may potentially be divided into two subspecies, the northern and southern provenances. This division was first suggested by Symon (1966), based on differences in leaf shape, which in the southern provenance of *C. brewsteri* approaches that of *C. marksiana*. Stanley and Ross (1983) distinguish the two forms of *C. brewsteri* on the basis of the moist forest form (southern) having larger leaflets with broader sutures. They also note, along with Turnbull *et al.* (1986), that the southern provenance is much taller (up to 30 m compared to 12 m).

An apparently unidentified specimen, *Cassia* sp. 'Paluma Range' (G. & N. Sankowsksy 450) was collected inland from Townsville and is also recorded on rare plant lists as occurring on Magnetic Island (Thomas and McDonald 1989, Briggs and Leigh 1995). This potential taxon probably represents an isolated population of *C. brewsteri*. All of the *Cassia* herbarium sheets in Australia were examined during the preparation of Flora of Australia treatment, and G. & N. Sankowsksy 450 did not attract special attention (Randell pers. comm.), hence it was not considered in the current study.

The aim of the current study was to assess the genetic diversity of *C. brewsteri* in the wider context of the taxonomy of Australian *Cassia*. Symon (1966) noted that *C. brewsteri*, *C. tomentella* and *C. marksiana* are often confused due to poor herbarium specimens which rarely combine leaves, flowers and pods. It is not clear whether the wide morphological variation in Australian *Cassia* is primarily due to intergradation between species, environmental influences or inherent genetic diversity. Here, we present the results of a taxonomic analysis based on molecular genetic markers and geographic distribution.

Materials and methods

Botanical sources and population sampling

The herbarium sheets for each of the four native *Cassia* species at the Queensland Herbarium (BRI) were examined prior to field collections. Herbarium specimen distributions (mainly BRI) and new collection sites were mapped in ArcView (ESRI 1996) and presented in Albers equal-area conic projection (Fig. 1.1).

C. brewsteri accessions (30) were collected from throughout the natural distribution of the species (Table 1.1). Three accessions of *C. tomentella* were selected along an east-west transect through a putative zone of intergradation with *C. brewsteri* (latitude c. 23° 27' south). In most cases seed was collected, and later germinated to obtain leaf material for DNA extraction. Where fresh leaf tissue was used from mature trees in the field, leaves were wrapped in damp newspaper or placed directly into 2 mL tubes and transported on ice to prevent decay. Single *C. queenslandica* and *C. marksiana* seedlots were provided by the Australian Tree Seed Centre, Canberra.

Seedlings were raised in a controlled temperature glasshouse in tubes and pots of various sizes. The youngest fully expanded leaflets available (200-300 mg), were collected and either immediately used for DNA extraction or stored in 2 mL tubes at -80 °C until DNA extraction.

When collecting leaf samples from wild trees, care was taken to select the youngest fully expanded leaflets that were free of insect eggs and larvae. Particularly common on *Cassia* were eggs of the migrant butterfly, *Catopsilia* spp. This presented difficulties when collecting late in the growing season because there were few new leaves forming and these were favourable for both DNA extraction and for insect oviposition and larvae.

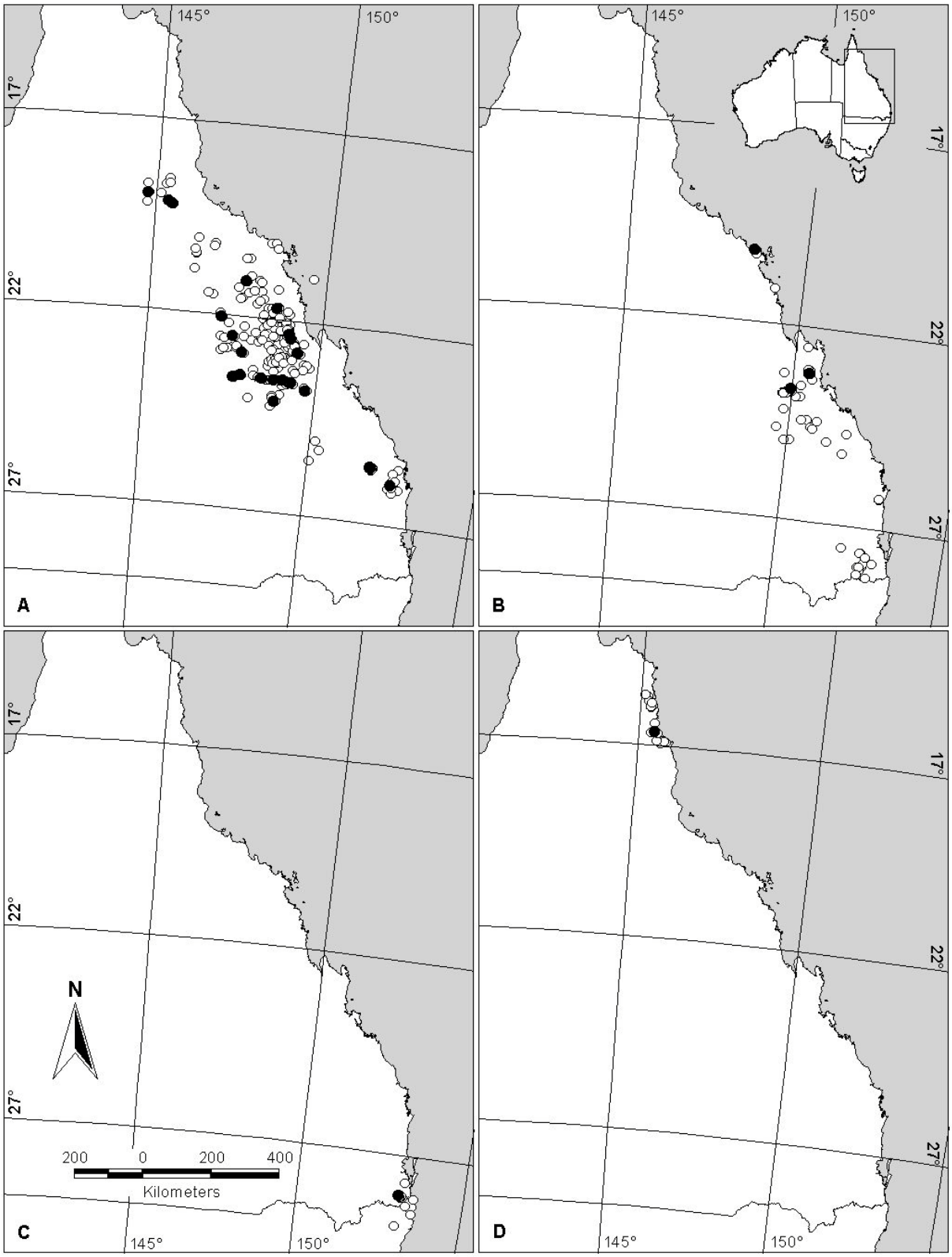

Figure 1.1. Natural distributions (hollow circles) and germplasm sample sites (solid circles) of **A.** *Cassia brewsteri* (30 accessions), **B.** *C. tomentella* (three accessions), **C.** *C. marksiana* (one accession) and **D.** *C. queenslandica* (one accession).

Table 1.1. *Cassia* accessions studied.

DNA sample	Latitude decimal° S	Longitude decimal° E
C. queenslandica	16.660	145.450
C. brewsteri 77C (N)	19.002	144.800
C. brewsteri 76A (N)	19.180	145.380
C. brewsteri 75A (N)	19.257	145.483
C. brewsteri 51A (N)	21.106	147.740
C. brewsteri 80A (N)	21.744	148.696
C. brewsteri 47A (N)	22.086	147.146
C. brewsteri 3B (N)	22.368	149.108
C. brewsteri 81A (N)	22.487	149.169
C. brewsteri 73A (N)	22.560	147.515
C. brewsteri 44A (N)	22.833	149.406
C. brewsteri 69A (N)	22.974	147.829
C. brewsteri 68D (N)	23.547	147.828
C. brewsteri 67E (N)	23.547	147.826
C. brewsteri 15D (N)	23.584	149.078
C. brewsteri 16D (N)	23.589	148.792
C. brewsteri 66D (N)	23.595	148.454
C. brewsteri 70A (N)	23.609	147.627
C. brewsteri 17B (N)	23.624	149.318
C. brewsteri L16 (N)	23.633	149.257
C. brewsteri L20 (N)	23.633	149.257
C. brewsteri L6 (N)	23.633	149.257
C. brewsteri L6m (N)	23.633	149.257
C. brewsteri L33 (N)	23.633	149.257
C. brewsteri L33m (N)	23.633	149.257
C. brewsteri L42 (N)	23.633	149.257
C. brewsteri DCC6m (N)	23.787	149.747
C. brewsteri 21C (N)	24.140	148.878
C. brewsteri 95A (S)	25.554	151.890
C. brewsteri 94A (S)	25.579	151.886
C. brewsteri The. m (S)	25.950	152.550
C. tomentella 120C	23.221	150.476
C. tomentella 45A	23.663	149.994
C. tomentella DCC7m	23.781	149.769
C. marksiana	28.050	153.100

N = northern provenance, S = southern provenance,
L = Longdale site, m = maternal leaf tissue

Genomic DNA isolation

Total genomic DNA was extracted after Doyle and Doyle (1990) with modifications. Fresh or frozen leaf tissue (200-300 mg) in a 2 mL tube was ground in liquid N using a plastic micropestle. Extraction buffer (1000 µL) was added to the homogenate in the tube (100 mM Tris-HCl, pH 8.0; 20 mM ethylenediaminetetraacetic acid (EDTA); 1.4 M NaCl; 2% (w/v) cetyltrimethylammonium; polyvinylpyrrolidone and 0.2% β-mercaptoethanol). After incubation at 65 °C for 45-60 min with slow inversion every 10 min, extracts were centrifuged at 10 000 rpm for 3 min. The supernatant was transferred to another 2 mL tube and washed with an equal volume of chloroform:isoamyl alcohol (24:1) by inverting slowly c. 100 times. The aqueous phase was recovered by centrifugation at 14 000 rpm for 3 min. DNA was precipitated by adding an equal volume of ice-cold isopropanol and incubating at -20 °C overnight. The DNA was pelleted by centrifugation at 14 000 rpm for 5 min, then washed twice with ice-cold 70% ethanol, dried at ambient temperature for c. 90 min and reconstituted with 20-30 µL sterile milliQ H_2O at 4 °C overnight.

RAF amplification

Randomly Amplified DNA Fingerprints (RAFs) has recently been developed from components of the earlier arbitrarily primed PCR protocols[1]. It most closely resembles the latest reported DAF protocol (DNA Amplified Fingerprinting) (Bentley and Bassam, 1996), but is considerably more sensitive because the amplicons are labelled with either α-labelled 33P or fluorescent tags, and are separated and detected using large polyacrylamide sequencing gels. The RAF protocol is highly reliable and efficient, and represents a culmination of the earlier marker protocols based on arbitrarily amplified markers.

Amplification of genomic sequences was performed after the RAF method, modified slightly for *C. brewsteri* with the addition of Bovine Serum Albumin (BSA) and the use of α-labelled ^{33}P-dCTP (in place of α-labelled ^{33}P-dATP). The PCR was conducted in 10 µL of DAF buffer (Caetano-Annolles *et al.* 1991 a, b) containing 0.02 mM each of dATP, dCTP, dGTP and dTTP, 2.5 µCi α-labelled ^{33}P-dCTP (Amersham), 200 ng BSA, 5 µM primer, 1.5 U *Taq* DNA polymerase, Stoffel fragment (Perkin Elmer) and 40 ng of high molecular weight template DNA. Reactions were prepared on ice and loaded into the thermal cycler (Corbett Research FTS-960 Thermal Sequencer) with the block at 85 °C. The thermal profile of the PCR included an initial strand separation step at 94 °C for 5 min, followed by 29 cycles of denaturation at 94 °C for 30 sec, touchdown annealing for 1 min at each of 57 °C, 56 °C, 55 °C, 54 °C and 53 °C, and then a final extension step at 72 °C for 5 min.

Electrophoresis conditions

The amplified products were denatured with the addition of 10 µL acrylamide gel loading buffer (98% formamide, 10 mM EDTA pH 8.0, 0.05% bromophenol blue and 0.05% xylene cyanol) and heating to 90 °C for 6 min in the thermal cycler. Samples (2 µL) were loaded onto a polyacrylamide (4%) denaturing gel (45% urea) and separated at 100 W for 2 hr 15 min in a nucleic acid sequencer (Hoefer Poker Face II SE 1600). An internal standard was run every five to six lanes and one negative control was run for each primer. The gel was cooled, lifted onto chromatography paper (46 × 57 cm), sealed on one side with plastic wrap, trimmed, then dried at 80 °C for 45 min under low pressure (Hoefer DrygelSR Slab Gel Dryer SE 1160). A sheet of one-sided autoradiography film (Kodak Biomax MR-1, 35 × 43 cm) was exposed to the dry gel in a cartridge (Amersham Hypercassette) for 16 to 48 hr depending on signal strength. Films were developed automatically in a medical film processor (Konica SRX-101).

The molecular weight range was estimated using *Hin*f1 digested *Phi*X174 DNA (Promega) kinased to γ-labelled ^{33}P-ATP with T4 poly-nucleotide kinase (NEB) in T4 buffer (250 mM Tris pH 7.5, 100 mM $MgCl_2$, 50 mM DTT, 5 mM Spermidine) at 37 °C for 60 min and 70 °C for 10 min. The kinased ladder was loaded in a solution of AFLP buffer and acrylamide loading buffer.

Selection of primers

Twelve primers (decamer oligonucleotides) were obtained from Advanced Biotechnologies (ABI) (equivalent to primers in the Operon Technologies 10-mer Kit B). After initial screening, larger quantities of primers were custom made by Gibco BRL, although the ABI nomenclature was retained for convenience. The twelve primers were screened with single accessions of *C. brewsteri* and *C. queenslandica* (Table 1.2). Each of the 12 primers generated PCR products with c. 15 to 30 bands showing polymorphism between *C. brewsteri* and *C. queenslandica*. Six primers were chosen for further experiments based on a compromise of maximum number of bands (for *C. brewsteri* DNA) and maximum number of polymorphisms (relative to *C. queenslandica* DNA).

[1] J. Waldron, M. Graham and B.J. Carroll, The University of Queensland, (personal communication).

Table 1.2. Primers screened and used for analysis of genetic diversity in Australia *Cassia.*

Name	Sequence (5'-3')	Name	Sequence (5'-3')
ABI-06*	TGC TCT GCC C	ABI-03*	CAT CCC CCT G
ABI-08*	GTC CAC ACG G	ABI-04*	GGA CTG GAG T
ABI-11*	GTA GAC CCG T	ABI-07*	GGT GAC GCA G
ABI-01	GTT TCG CTC C	ABI-09	TGG GGG ACT C
ABI-02	TGA TCC CTG G	ABI-10	CTG CTG GGA C
ABI-05	TGC GCC CTT C	ABI-12	CCT TGA CGC A

* Indicates primers chosen for further experimentation.

Data analysis

Each RAF profile was analysed visually over a light table. The presence or absence of each polymorphic band, from c. 70 to 500 kb, was recorded as a '1' or a '0' respectively. Matrixes of dissimilarity (1-S) were generated by the program RAPDPLOT version 3 (Black 1997) using the matching coefficient method of Nei and Li (1979). In this method the similarity index (S) of each pair-wise comparison of individuals is calculated as:

$$S_{AB} = 2 \times N_{AB} \div (N_A + N_B)$$

N_{AB} is the number of common bands shared by individuals A and B, N_A is the number of bands recorded for an individual A and N_B is the number of bands recorded for individual B. Only polymorphic bands, across the profiles of all accessions, are scored in this type of analysis.

Multidimensional scaling (MDS) was used to produce two-dimensional ordinations of the data from the dissimilarity matrixes. Monotonic MDS with minimising Guttman/Lingoes coefficient of alienation was performed in SYSTAT version 9 (SPSS).

A separate cluster analysis was conducted using several programs in PHYLIP (PHYLogeny Inference Package) version 3.5c (Felsenstein 1993). The bootstrapping function of RAPDPLOT was used to generate 100 pseudoreplicated data matrices, which were entered into NEIGHBOUR to produce 100 treefiles, using the Unweighted Pair Group Method with Arithmetic Averages (UPGMA). CONSENSE was used to calculate a consensus treefile and a set of bootstrap values for each node. The phenograms were produced by entering the treefile from NEIGHBOUR into RETREE then DRAWGRAM to produce a graphical output. The bootstrap values were manually transcribed to the appropriate node of the phenogram using data from the CONSENSE outfile.

Results

DNA isolation and generation of RAF profiles

Isolation of high molecular weight DNA with minimal shearing from the native *Cassia* proved difficult. In most cases several extracts were prepared in order to obtain two that could be used for RAF analysis. Duplicate DNA extractions from individual plants gave identical DNA profiles (data not shown). Each sample produced a RAF profile that could be scored in the range of 70 to 500 kb (representative RAF profiles, Fig. 1.2). BSA was essential for generation of a useful RAF profile with most of the *C. brewsteri* DNA extracts and was used for all samples to maintain uniformity of methods.

Genetic diversity of Australian Cassia

Three primers (ABI-06, ABI-08 and ABI-11) were sufficient to generate 265 scorable polymorphic markers among the 35 accessions. Three additional primers (ABI-03, ABI-04 and ABI-07) were used for a separate six-primer analysis of nine representative accessions based on 393 polymorphisms.

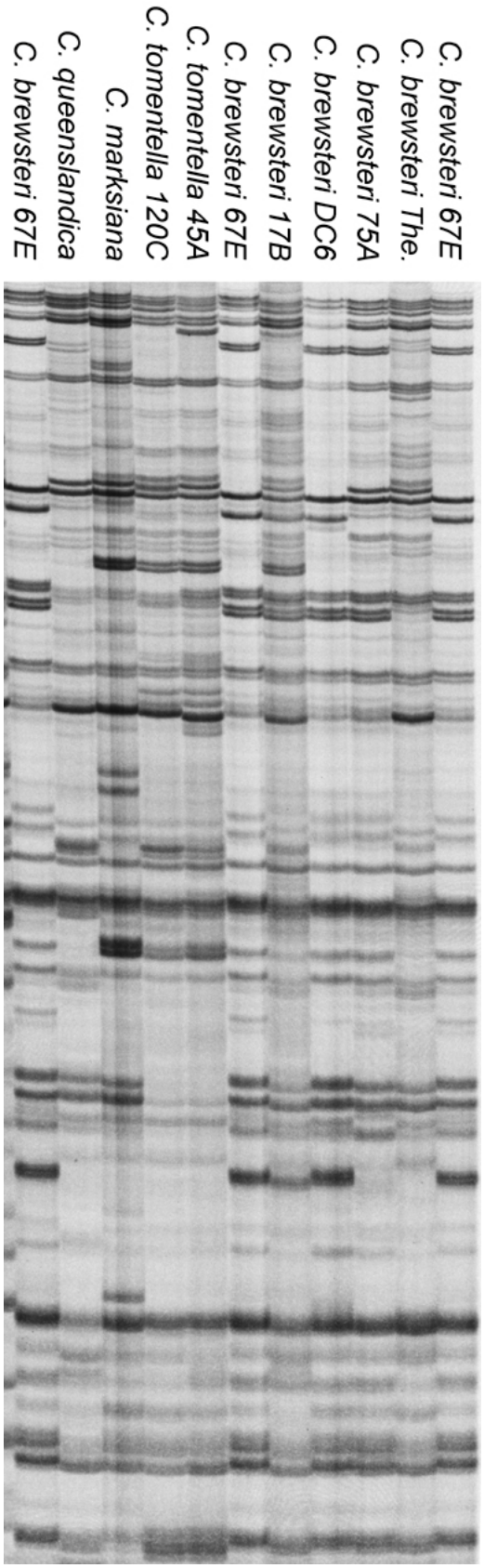

Figure 1.2. Comparison of Australian *Cassia* species using Randomly Amplified DNA Fingerprints with primer ABI-06.

The dissimilarity matrices generated by scoring the RAF profiles were used for the MDS and phenogram analyses. The three-primer analysis separated *C. brewsteri* into northern and southern clusters distinct from separate positions occupied by *C. queenslandica*, *C. tomentella* and

C. marksiana (Fig. 1.3). The differentiation between the northern and southern *C. brewsteri* provenances was comparable to that among the other taxa. Accession 17B was positioned in between *C. brewsteri* (north) and *C. tomentella*. This accession was collected from a population located near the eastern border of the distribution of *C. brewsteri* at the latitude of c. 23.5° S. The leaf and pod characters of the maternal plant were intermediate between *C. brewsteri* and *C. tomentella* (lower leaflets almost orbicular, pods almost cylindrical and sparingly pubescent). The flowers of the maternal plant were pure yellow, a character ubiquitous in *C. tomentella* but rare in *C. brewsteri*. The seedling raised for the RAF analysis also had orbicular lower leaflets and may represent an *n*th generation hybrid of the two species.

The six-primer MDS analysis of nine representative native *Cassia* accessions also separated *C. queenslandica* from the other three taxa (Fig. 1.4). *C. brewsteri* clustered into northern and southern provenances, with 17B split from the other northern accessions. The *C. tomentella* accessions segregated strongly from *C. marksiana*.

The phenogram of the nine accessions based on 393 polymorphisms (six primers) produced the same basic topology as the two MDS analyses (Fig. 1.5). *C. queenslandica* was on an outlying branch. The three *C. brewsteri* accessions segregated together with the southern provenance an outlier. *C. tomentella* and *C. marksiana* clustered together, with *C. marksiana* distinguished from the three *C. tomentella* accessions. Bootstrapping support for each node was 100% with the exception of the sub-clusters within the *C. tomentella* branch.

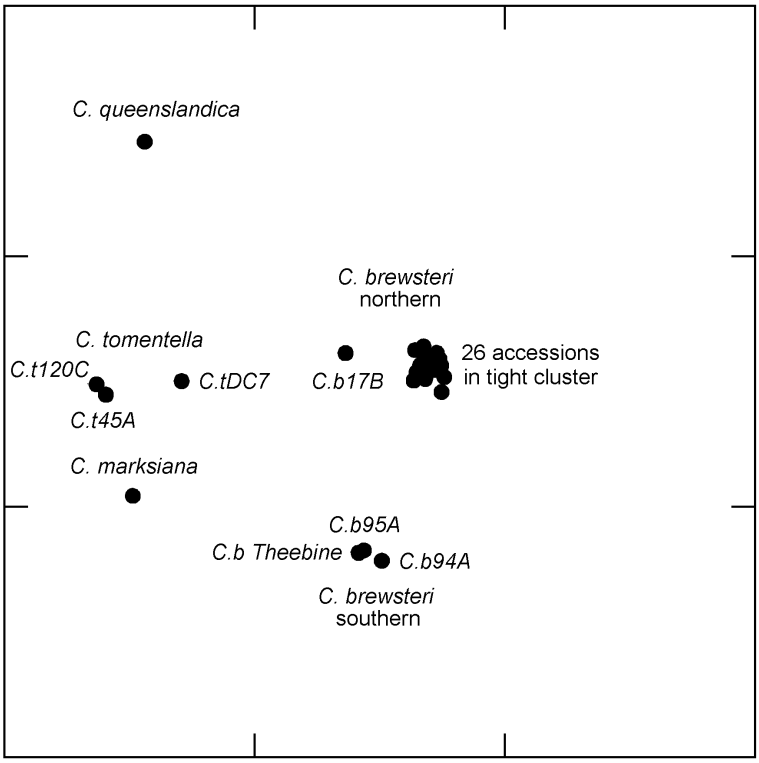

Figure 1.3. Multidimensional scaling ordination plot of the genetic similarities between 35 Australian *Cassia* accessions based on 265 RAF polymorphisms generated by three primers. Alienation of final configuration: 0.09067, proportion of variance (RSQ): 0.99025.

Discussion

Taxonomy of Australian *Cassia*

The MDS analyses of genetic similarities strongly support the maintenance of *C. brewsteri*, *C. tomentella*, *C. marksiana* and *C. queenslandica* at species level after Symon (1966). Further, Symon's (1966) suggestion that *C. brewsteri* may consist of two subspecies is supported. Indeed the genetic distance between the northern and southern *C. brewsteri* provenances is comparable to the

differentiation between each provenance and the other three *Cassia* species. These conclusions contrast with Randell and Barlow's (1998) treatment, which reduced *C. brewsteri*, *C. tomentella* and *C. marksiana* to varieties of a single species, and did not distinguish sub-varieties of *C. brewsteri* var. *brewsteri*.

The phylogenetic relationships among the four major taxa remain uncertain. Symon's (1966) association of *C. brewsteri* with *C. queenslandica* is not supported by the phenogram shown here which separates *C. queenslandica* from the other taxa. The MDS plots also place *C. queenslandica* as an outlier. The most likely phylogeny is therefore a combination of those of Symon (1966) and Randell and Barlow (1998), similar to that presented in Fig. 1.5. The four major taxa probably represent different species but *C. brewsteri*, *C. tomentella* and *C. marksiana* are more closely related to each other than they are to *C. queenslandica*.

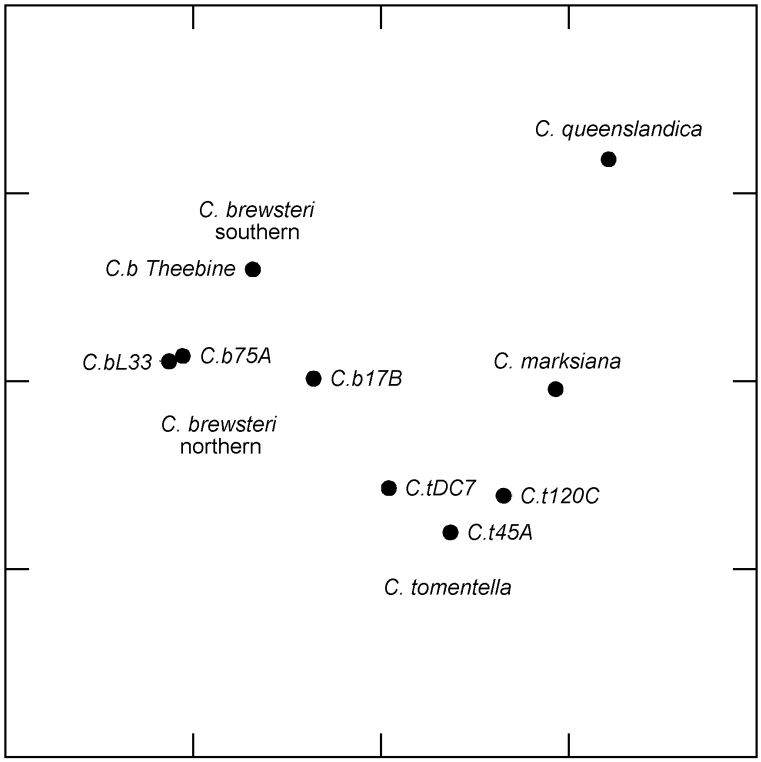

Figure 1.4. Multidimensional scaling ordination plot of the genetic similarities between nine Australian *Cassia* accessions based on 393 RAF polymorphisms generated by six primers. Alienation of final configuration: 0.05038, proportion of variance (RSQ) is: 0.99442.

Hybridisation of *Cassia brewsteri* and *Cassia tomentella*

Individuals at site 17 were identified as *C. brewsteri* but featured some morphological characteristics diagnostic of *C. tomentella*. The MDS analysis split accession 17B from the *C. brewsteri* northern ssp, and positioned the accession in between *C. brewsteri* and *C. tomentella*, but closer to *C. brewsteri*. These results suggest hybridisation between the two species, followed by introgression to 'pure' *C. brewsteri*.

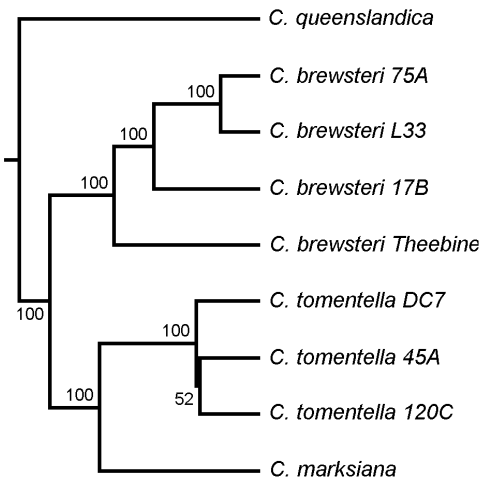

Figure 1.5. Consensus tree of nine Australian *Cassia* accessions based on 393 RAF polymorphisms generated by six primers.

Conservation issues

The taxonomic level is significant for conservation of species since conservation programs are generally focussed on species as a whole and not subspecific taxa. No *Cassia* species are currently listed as endangered in the Australian and New Zealand Environment and Conservation Council's 'List of Threatened Australian Flora' (ANZECC 1997).

C. brewsteri is present in large numbers throughout the cattle grazing areas of central Queensland, as far inland as Anakie. It is commonly cleared in these regions as it is considered a weed. The plant is represented in conservation reserves including Scawfell Island and Dipperu National Parks. No studies of the impact of clearing on the species biodiversity are known.

C. marksiana and *C.* sp. 'Paluma Range' are ROTAP listed (Rare or Threatened Australian Plants) (Briggs and Leigh 1995). *C. queenslandica*, *C. marksiana* and *C.* sp. 'Paluma Range' are all listed as rare in Thomas and McDonald (1989). *C. marksiana* and C. sp. 'Paluma Range' are both coded 2RC because of limited natural distribution (2), rarity (R) and representation within a reserve (C). *C. queenslandica* is classed 3R i.e. it has a range greater than 100 km (3) but remains rare (R) because it occurs in small populations.

If *C. marksiana* was officially recognised as a distinct species, a strong case could be made for listing it as endangered. *C. marksiana* is in a precarious position since it has been much reduced by clearing and is now rare (Stanley and Ross 1983, Hauser 1992). It is reportedly conserved in Lamington and Nicoll Scrub National Parks and Currumbin Environmental Park (Forster 1991). However, in 1989 there were only 13 trees left in the wild in NSW, several of which were in a senile state (Floyd 1989). According to the label of two specimens in the Coffs Harbour Botanic Gardens in 1999, there are only about 30 trees left in the wild in total.

2. Ecological requirements of *Cassia brewsteri*

Abstract

The natural distribution of *Cassia brewsteri* was determined by compilation of data from herbaria and from field studies. The ecological parameters of natural occurrences were characterised in terms of soil and vegetation types by sampling 113 sites throughout the natural distribution in Queensland (latitudes 18.583 - 26.150° S, longitudes 144.750 - 152.750° N). In addition to natural occurrences, a further 11 sites were identified where the plant had successfully been grown in cultivation. The natural and cultivated distributions were used as a basis for predicting potential sites for cultivation of the tree using the climate modelling software BIOCLIM. The results show a broad area of eastern Queensland where the tree may be grown for seed gum production under irrigation. Failures of the tree in agroforestry trials in Thailand may be explained by high temperature being a limiting factor in the distribution of the species. Failures of the species in agroforestry trials in Australia, Zimbabwe and Malawi could not be attributed to climate or soil conditions at the trial sites indicating that further research on culture of the plant is required to achieve high survival and growth rates.

Introduction

Cultivation of *Cassia brewsteri*

Although *C. brewsteri* has been used with success as an ornamental and shade tree, experimental agroforestry trials of the species have generally failed due to poor growth performance. *C. brewsteri* has been included in five agroforestry trials conducted on eight sites in four countries. These include two sites in Queensland (Ryan and Bell 1989), one site in Malawi (Maghembe and Prins 1994), three sites in Thailand (Pinyopusarerk 1989) and two sites in Zimbabwe (Gwaze 1989, Mitchell 1989). The growth performance of *C. brewsteri* in all of the trials reported was among the worst of all the species grown for all of the parameters measured (Table 2.1).

Table 2.1. Summary of growth performance for *Cassia brewsteri* trials.

Country/site	Age (months)	Survival (%)	Mean height (cm)	Mean diameter at ground (cm)
Australia				
Tuan	31	84	82	1.7
Wongi	31	failed	failed	failed
Thailand				
Ratchaburi	12	28.9	43	1.15
Si Sa Ket	12	56.1	59	1.13
Chiang Mai	12	29.0	10	0.33
Zimbabwe				
Makoholi	18	failed	failed	failed
Kadoma	18	50.7	35	not reported
Kadoma 2	18	86.4	45	2.84
Malawi				
Makoka	26	44	failed	failed

Sources: Australia (Ryan and Bell 1989), Thailand (Pinyopusarerk 1989), Zimbabwe (Gwaze 1989, Mitchell 1989) and Malawi (Maghembe and Prins 1994).

In an Australian Centre for International Agricultural Research (ACIAR) trial reported by Ryan and Bell (1989), *C. brewsteri* was included with 177 Australian species on plots in Tuan and Wongi State Forests near Maryborough in south east Queensland. Pinyopusarerk (1989) reported the Thailand results of the same ACIAR trial in which *C. brewsteri* was included in trials on three of seven sites, Ratchaburi, Si Sa Ket and Chiang Mai. In the ACIAR trial in Zimbabwe, *C. brewsteri* was included on two of the five sites, Makoholi and Kadoma (Gwaze 1989). A second trial conducted at Kadoma

(Mitchell 1989) investigated the differences between species in terms of susceptibility to termite attack. The performance of *C. brewsteri* in this trial was marginally better than the first Kadoma site with no mortality attributed to termites. The seedlot used in the termite susceptibility trial was different, although the seedlot was from the same region, Blackwater in central Queensland to the other trials of *C. brewsteri*. The improved growth performance was probably because this particular trial was watered on four occasions during a period of low rainfall.

Maghembe and Prins (1994) report a trial of 37 multipurpose trees for agroforestry in Malawi, southern Africa. This trial used the same Australian Tree Seed Centre (ATSC) seedlot as the termite resistance trial at Kadoma, Zimbabwe. Had these trials been successful there would be the opportunity to assess the yield of *C. brewsteri* in terms of fruit, seed and gum when grown under different conditions. Unfortunately, the reasons for the failure of the species in these trials were not analysed by the respective authors.

Distribution

Symon (1966) and Forster (1991) have produced distribution maps based on actual herbarium records. The number and distribution of the collections available at the time and the number of collections used limit the value of these maps respectively. Forster (1991) used only four collections and only considered the vineforest distributions i.e. in the region surrounding the southern part of the distribution of the plant. The map of Symon (1966) features 15 points which cover more of the range of the species but are not representative of the whole range. The Queensland Herbarium (BRI) database (HERBRECS) features a map output that can display all of the collections that have been entered into the database, this database currently provides an adequate coverage of the range of *C. brewsteri*. Turnbull *et al.* (1986), Anderson (1993) and Randell and Barlow (1998) have produced distribution maps based on previously known distributions and personal knowledge of the plant. Anderson's (1993) map accurately represents the borders of the plant's two main populations while Randell and Barlow's (1998) map is an inaccurate random swathe across eastern Queensland.

Aim of the study

Site selection for groups of many species including *C. brewsteri* has been addressed in general texts on cultivation of Australian native plants for amenity horticulture. For example, Rowell (1991) recommends *C. brewsteri* be planted on light, freely-drained soils in the climatic regions of Sydney, Brisbane and the tropics. This type of information is too general for decision support for agricultural applications. The aim of this report is to produce a comprehensive ecological description for *C. brewsteri* which, combined with previous work, may be applied to site selection for cultivation of the tree.

Materials and methods

Natural distribution

Natural distributions and other information were extracted from unpublished data collected between 1978 and 1980 for a study of woody plant species in central Queensland pastures undertaken by the Queensland Department of Primary Industries (DPI) (Anderson *et al.* 1984). Information from sites where *C. brewsteri* occurred was obtained by examination of the original field datasheets from 460 sites covering 357,000 ha. The latitude and longitude records on the DPI datasheets had been determined from the 1:100,000 map series and recorded in degrees and minutes. In some cases the locality description and property name were able to clarify the position of Queensland Herbarium (BRI) collections where ambiguous locality descriptions or property names led to inaccuracies in latitude and longitude estimates, particularly in older collections. Further records were obtained from Anderson's unpublished observations of native plants recorded from 1977 to 1999.

The BRI records for *C. brewsteri* were extracted from HERBRECS on 21 April 1997. Latitude and longitude records were provided for most collections, ranging in accuracy from no latitude or longitude to a GPS record made by the collector. Where there is no accurate position provided by the collector, an estimate is made by the Herbarium staff based on the locality description provided by the

collector. The positional accuracy of localities, in minutes, was zero, one or two significant figures. Where two significant figures were provided the data were converted directly to decimal degrees and imported into the distribution data. Where zero or one significant figure was provided the latitude and longitude were determined on the basis of the locality description using the gazetteers of Anon. (1975) and AUSLIG (1995), and the maps of Aplin *et al.* (1994). Where the position could not be plotted within ± 10' due to the vagueness of the locality description, the record was not used for distribution mapping.

Climate modelling and site matching

The distribution records were geocoded by assigning a latitude, longitude and elevation to each unique site. The elevations of each distribution were determined by interrogation of a 30 arc second Digital Elevation Model (DEM), GTOPO30, produced by the EROS Data Centre of the United States Geological Survey (USGS 1997). Elevations were extracted using a script downloaded from the ESRI ArcScripts website (http://gis.esri.com/arcscripts/scripts.cfm).

The geocoded information was analysed with the software package ANUCLIM (Hutchinson *et al.* 1998). Bioclimatic profiles were generated based on the confirmed distributions in Australia using BIOCLIM and ESOCLIM. Selected parameters from these profiles were compared with the climate profile for the entire continent using the BIOMAP element of ANUCLIM. The results were mapped in ArcView (ESRI 1996) and presented in Albers equal-area conic projection.

Cultivated distribution

Cultivated records were obtained from HERBRECS, published literature, personal communications and field surveys. An article requesting information was also placed in the Society for Growing Australian Plants (SGAP) Queensland newsletter (December 1998), resulting in two detailed replies.

The Australian Tree Seed Centre (ATSC) maintains collections of native tree seeds for use in forestry programs throughout the world. ATSC records showed 17 seed dispatches to 15 customers in six countries from 1984 to 1995. Letters to ATSC clients yielded information from one unpublished study (Ashwath and Marcar n.d.) but no additional field trials.

Population sampling

Forty field sites were located in 1998 representing the geographical range of the species determined using the methodology described above. An attempt was made to extend the known distribution by travelling an additional c. 50 km past the edge of the known distribution in several places. The position of each site was determined using a handheld Global Positioning System (GPS) receiver (Garmin) without differential correction. A locality description was recorded using the vehicle odometer to note the distance from the nearest town.

Vegetation characterisation

Original vegetation communities were described in the field on the basis of remnant vegetation and regrowth, vegetation structure was classified after Specht (1970). Site disturbance was recorded as one of seven categories after Pressland (c. 1992) i.e. (1) no disturbance, (2) ringbarking, (3) injection, (4) pulling, (5) stickraking, (6) blade-plough and (7) other.

Information from the unpublished datasheets used in the report of Anderson *et al.* (1984) was incorporated into the results for vegetation communities and site disturbance. Coppicing ability was quantified from the unpublished datasheets used in the report by Anderson *et al.*, based on records of re-growth height and known time of clearing.

Growth habit

At each site, the number of stems was counted and the height and minimum and maximum crown diameter were recorded for up to five trees using a graduated measuring stick. An average crown

diameter was estimated by taking the square root of the product of the minimum and maximum crown diameters.

Landscape

The landscape of each site was characterised according to slope, aspect and topography. Slope was measured with a clinometer, aspect with a compass and the topography was classified according to a seven-point scale after Pressland (c. 1992). Information from the unpublished datasheets used in the report of Anderson *et al.* (1984) was incorporated for slope and topography.

Soil characterisation

Soil samples were taken at 0-10 cm and 40-50 cm from a single core and the pH and EC analysed after Rayment and Higginson (1992). Soil texture was estimated in the field by hand ribboning after Northcote (1979) and changes were noted down the soil profile to a depth of 50 cm. Information from the unpublished datasheets used in the report of Anderson *et al.* (1984) was incorporated for soil texture to 90 cm, pH at surface and pH deeper. A basic description of soil colour was also recorded in the field.

Results and discussion

Natural Distribution

A total of 310 distribution records were determined. Many herbarium records represented the same site collected from on more than one occasion, or different sites that could not be differentiated on the basis of the locality description (e.g. "Blackwater"). A total of 248 points were obtained which were unique to three decimal places in decimal degrees i.e. the approximate accuracy of the non-differential GPS with selective availability.

The species occurred in the latitude range 18° 35' S to 26° 09' S and from the east coast of Queensland to c. 350 km inland (Fig. 2.1). The latitudes of the naturally occurring sites sampled for ecological data ranged from 19° 00' S to 25° 58'S. Of the 460 sites sampled for the report produced by Anderson *et al.* (1984), 73 included *C. brewsteri* and these ranged in latitude from 21° 03' S to 23° 10'S.

Elevations for all sites ranged from near sea level (4 m) to 640 m. The highest elevation produced by the DEM matched the highest elevation of the BRI specimens (640 m). This result is in disagreement with the estimate of Turnbull *et al*. (1986) of c. 800 m.

Cultivated distribution

Eleven unique locations were identified where the plant has been grown in cultivation with at least some success. The BRI database included four collections of *C. brewsteri* in cultivation from 1966 to 1991. One locality was described only as Brisbane and was not used, as more precise locations within the Brisbane area were available. Two locations were reported by SGAP members in response to the request for information, four sites were located during field work and the two trial sites near Maryborough reported by Ryan and Bell (1989) were included after evaluation in 1998, i.e. at age 11.

Wrigley and Fagg (1979) record the plant in cultivation as far south as Sydney c. 33.5° S, and Elliot and Jones (1982) extend that range to Melbourne c. 37.5° S (in a protected position). Randell and Barlow (1998) include a floral illustration from a specimen of *C. brewsteri* in cultivation at the Waite Institute in South Australia. These locations were not included because the plant is thought to be cold sensitive and only moderately resistant to frost (Williams n.d.). Turnbull *et al.* (1986) estimate that in the natural range of *C. brewsteri* there are only between zero and four frosts per year.

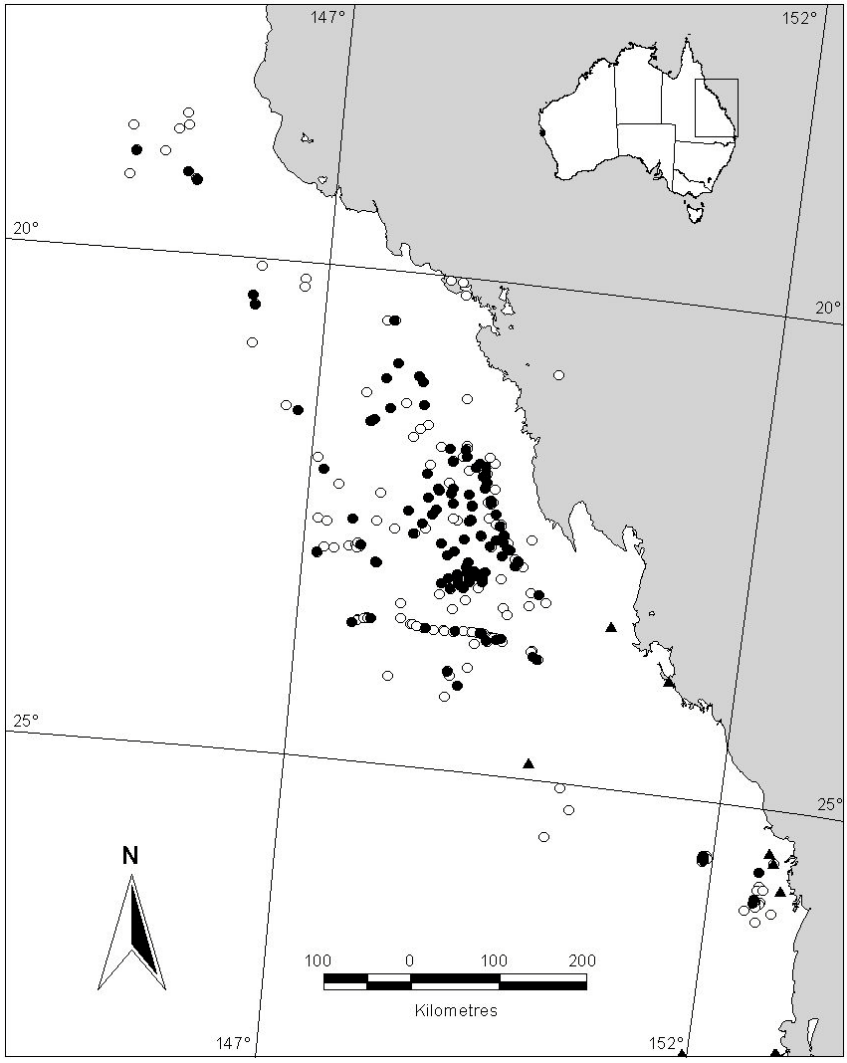

Figure 2.1. Distribution of *Cassia brewsteri* showing 248 natural locations (circles) and 11 cultivated locations (triangles) and 113 sites that were studied in detail (solid circles).

Vegetation structure and growth habit

C. brewsteri occurred mainly in woodland, open woodland and open forest with rare occurrences in tall open forest in the southern part of the distribution. The woodland and open forest communities were dominated by *Eucalyptus populnea* (poplar box), *E. brownii* (Reid River box), *E. crebra* (narrow-leaved ironbark), *E. melanophloia* (broad-leaved ironbark) or *Acacia harpophylla* (brigalow). In tall open forest, *C. brewsteri* occurred with *Araucaria cunninghamii*, *Flindersia australis*, *Eucalyptus siderophloia* and *E. maculata*.

Three main growth habits of the tree were observed; single-stemmed with a spreading crown, multi-stemmed with a spreading crown and multi-stemmed root suckers regenerating after clearing (Fig. 2.2). Single-stemmed specimens were relatively rare and ranged in height from 2.8 m to c. 30 m. The taller individuals were all located in the southern part of the distribution. In tall open forest the crown height was a small proportion of the tree height, c. 20%, while in all other vegetation structures the crown height exceeded c. 80% of the tree height.

C. brewsteri regenerates well after clearing and was commonly found as a multi-stemmed root sucker in cattle-grazing regions. The number of stems ranged from two to 24. No small seedlings (< 2 m) were observed, all smaller individuals were root suckers. Regrowth data were extracted from DPI datasheets for sites that were pulled or pulled and burnt (Table 2.1). Regrowth rates were similar following either clearing treatment. Growth rates observed at a given site will be influenced by water

availability, soil fertility and insect herbivory. A regrowth height of 1 m or more in a single season was commonly observed on recently cleared land during the collection of data for the present study.

Table 2.1. Regrowth rates for *Cassia brewsteri* after clearing.

Time since clearing (years)	Regrowth height (cm)	Height increment (cm/year)
1	80	80.0
2	80	40.0
6	200	33.3
6	100	16.7
7	90	12.9
7	130	18.6
7	60	8.6
8	180	22.5
10	150	15.0
12	200	16.7
15	120	8.0

Landscape

C. brewsteri occurred on flat alluvial plains, flat non-alluvial plains, plains with low relief, undulating plains with low rises and on low hills (Table 2.2). It did not occur on hilly to mountainous terrain or on tablelands despite these topographies being within the geographic range of the plant. Slope ranged from flat to 13° (n = 113). Aspect ranged from 20° to 340° (n = 17 non-flat sites).

Table 2.2. Frequency of topography classes for 113 natural distributions of *Cassia brewsteri*.

Topography class	Average slope (%)	Maximum slope (%)	% of records
plains flat, alluvial	0.5	<1	9.7
plains flat, not alluvial	0.5	1	31.0
plains with low relief	<3	3	25.7
plains, undulating with low rises	<9	12	25.7
hills, low	<18	24	8.0
hilly to mountainous	24	no limit	0
tablelands	-	-	0

Soil characterisation

C. brewsteri occurred on uniform, gradational and duplex soils, and on a range of soil textures (Table 2.3). Soil pH varied within each primary profile form, ranging from 4.2 to 9.0. Diemer and Simms (1996) report a maximum soil pH for *C. brewsteri* of 6.5 and an optimal pH of 6.0, their figures are very low compared to the majority of natural distributions of the plant, which are on neutral or alkaline soils.

The Electrical Conductivity (EC) of the A horizon ranged from 0.023 to 0.410 dS/m and the EC deeper in the soil profile was similar. Most occurrences were on relatively saline soils (Table 2.3), quantifying Turnbull *et al's.* (1986) assertion that the plant occurs on saline sites. In a study of salinity tolerance of 60 Australian tree species, *C. brewsteri* seedlings were found to be moderately salt tolerant, with an LD_{50} of 975 mol/m^3 and height growth ceasing at 640 mol/m^3 (Ashwath and Marcar n.d.).

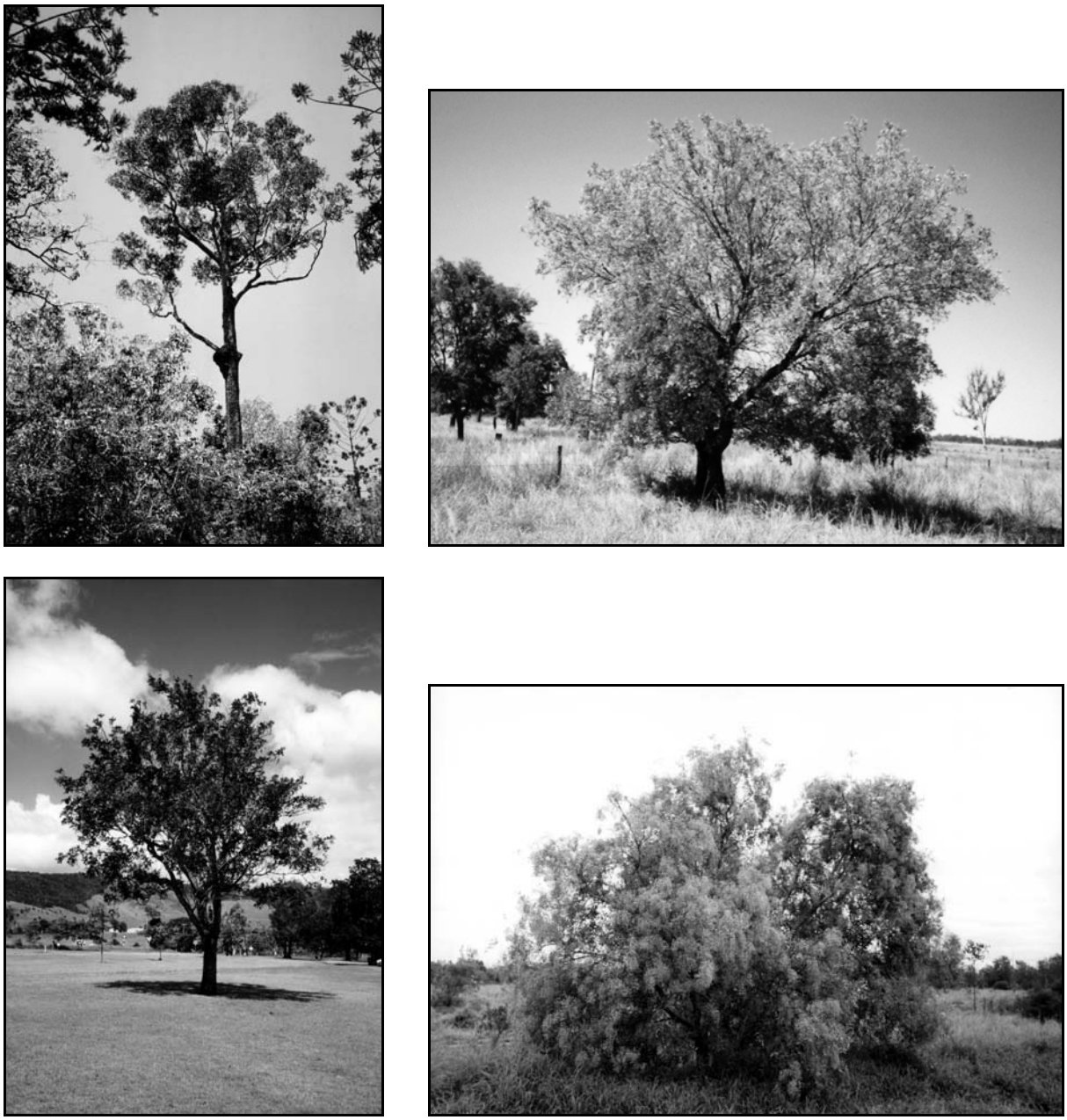

Figure 2.2. Various growth habits of *Cassia brewsteri*. Clockwise from top left: southern spp. mature straight tree in tall open forest (c. 30 m), northern ssp. mature spreading tree (c. 8 m), northern ssp. regrowth from root suckers in a blade-ploughed paddock (c. 4 m) and southern ssp. mature tree on a cleared periurban site.

Turnbull *et al.* (1986), later updated in Doran *et al.* (1997) reported that inland occurrences of *C. brewsteri* were commonly on alluvial plains and undulating lowlands with a permeable loam at the surface and an impermeable, often alkaline, medium to heavy clay subsoil. Other occurrences were reported to be on undulating to hilly country and tablelands where soils range from shallow skeletal sands and stony clays to deep loamy and sandy red earths. The best development of the tree was reported on deep, fertile red clay loams or deep alluvial soils near watercourses.

Table 2.3. Range and frequency of soil types for natural distributions of *Cassia brewsteri*.

Parameter	Results	% of sites
Primary profile form (n = 101)		
Uniform soils	Sandy loam to heavy clay (A horizon)	31.7
Gradational soils	Sandy clay loam to light medium clay (A horizon)	5.9
Duplex soils	Clayey sand to sandy clay (A) / clay loam to heavy clay (B)	62.3
Colours (A horizon) (n = 32)	Greys	15.6
	Browns	56.3
	Reds	28.1
pH (0 - 10 cm) (n = 97)	4.8 to 9.2	100
	Very acid (pH < 4.0)	0.0
	Acid (pH 4.0 – 6.0)	21.6
	Neutral (pH 6.1 – 7.4)	53.6
	Alkaline (pH > 7.4)	24.7
pH (≥ 40 - 50 cm) (n = 95)	4.2 to 9.0	100
	Very acid (pH < 4.0)	0.0
	Acid (pH 4.0 – 6.0)	9.5
	Neutral (pH 6.1 – 7.4)	25.3
	Alkaline (pH > 7.4)	65.3
EC (dS/m) (0 - 10 cm) (n = 26)	0.023 to 0.410	100
	Low (EC < 0.16 dS/m)	50
	High (EC > 0.16 dS/m)	50
EC (dS/m) (40 - 50 cm) (n = 24)	0.025 to 1.339	100
	Low (EC < 0.16 dS/m)	29.2
	High (EC > 0.16 dS/m)	70.8

Site selection modelling

BIOCLIM and ESOCLIM climate profiles were generated for (a) the 248 natural distribution of *C. brewsteri* and (b) these 248 plus the 11 cultivated distributions (Tables 2.5 and 2.6 respectively). The predicted distribution under rainfed conditions (Fig. 2.3) is similar to the actual distribution of the plant in nature.

The potential distribution under irrigation covers much wider area including a large part of eastern Queensland and a large belt through southern Australia (Fig. 2.4). These results indicate that the limiting factor in the western and northern distribution of the plant is higher temperature.

Limiting factors in *Cassia brewsteri* cultivation

The climate and soil conditions at *C. brewsteri* trial sites are summarised in Tables 2.7 and 2.8. The data for the overseas sites were obtained from publications of the trials while the Australian sites were described using the methodology of the present study. The climate profile for each of the trial sites matched the climate profile for *C. brewsteri* (Table 2.4) in most cases. The only exceptions were the higher mean annual temperatures at the three Thailand sites. This supports the conclusion that higher temperatures limit the natural distribution of the species in northern Australian (Fig. 2.4).

The data available for soils at the reported trial sites is even less complete than the climatic data. For the two Australian sites, the seven parameters assessed in the current study matched the profile for the natural distribution of the species with the exception of a slightly lower EC deeper in the profile at the Wongi site. No conclusions can be made with regard to soil limitations for the species due to the limited data available for trials of the species.

The species has failed in trials on sites with broadly similar soil and climate conditions to the natural range of the species. In some cases higher temperatures may have been a limiting factor but the problem could also be due to inappropriate management practices. Seedlings are variable in vigour and prone to multi-stemming; Mitchell (1989) reported a mean number of stems of 2.08 for a trial plot at 18 months in Zimbabwe. This may explain poor height growth but not poor basal area and volume

increment, which in this case were 0.26 cm^2 and 0.044 dm^3, respectively. Further work is required to identify optimal cultivation techniques for the species. Vegetative propagation has the potential to produce better, more consistent results if superior cultivars could be developed for orchard plantings, i.e. fast-growing trees with a single basal stem.

A number of other approaches have been developed for site selection specifically for forest trees, as recently reviewed by Booth (1991 and 1996), and Boland (1997). Whilst the detailed climatic profiles generated by BIOCLIM are useful for some applications, a simpler approach is often more useful for predicting suitable sites for forest trees. One of the earlier computer-based systems for selection of tree species for particular sites was the INSPIRE system of Webb *et al.* (1980). INSPIRE searched a database of tropical species and selected those which matched a profile of given climatic conditions.

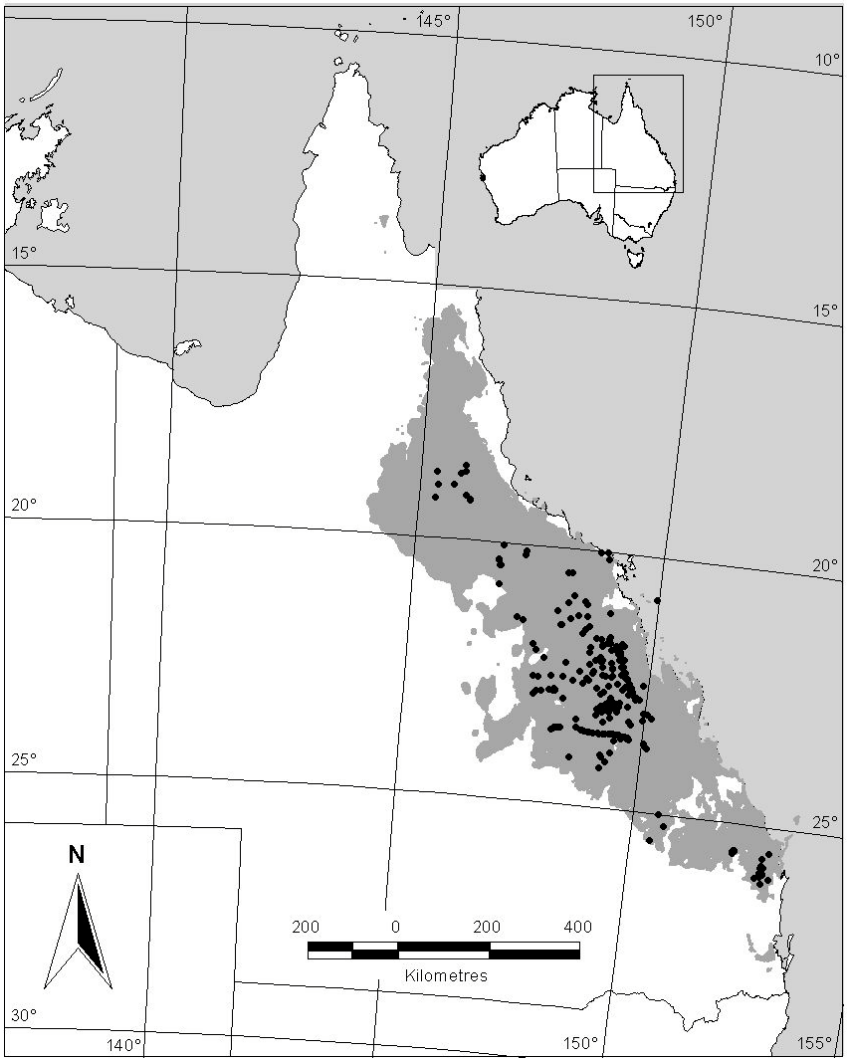

Figure 2.3. Areas suitable for cultivation of *Cassia brewsteri* under rainfed conditions. BIOMAP output showing limitations based on natural distribution and four Bioclimatic parameters.

Mean annual rainfall (mm)	509 - 1616
Mean maximum temperature of the hottest month (°C)	29.0 - 34.9
Mean minimum temperature of the coldest month (°C)	4.7 - 14.8
Mean annual temperature (°C)	19.1 - 23.4

Six of the climatic parameters used by INSPIRE were selected for use in the simulation mapping programs (e.g. GROMAP) developed in Australia in the 1980s for forest trees. These are listed in Table 2.4 along with data derived from the present study.

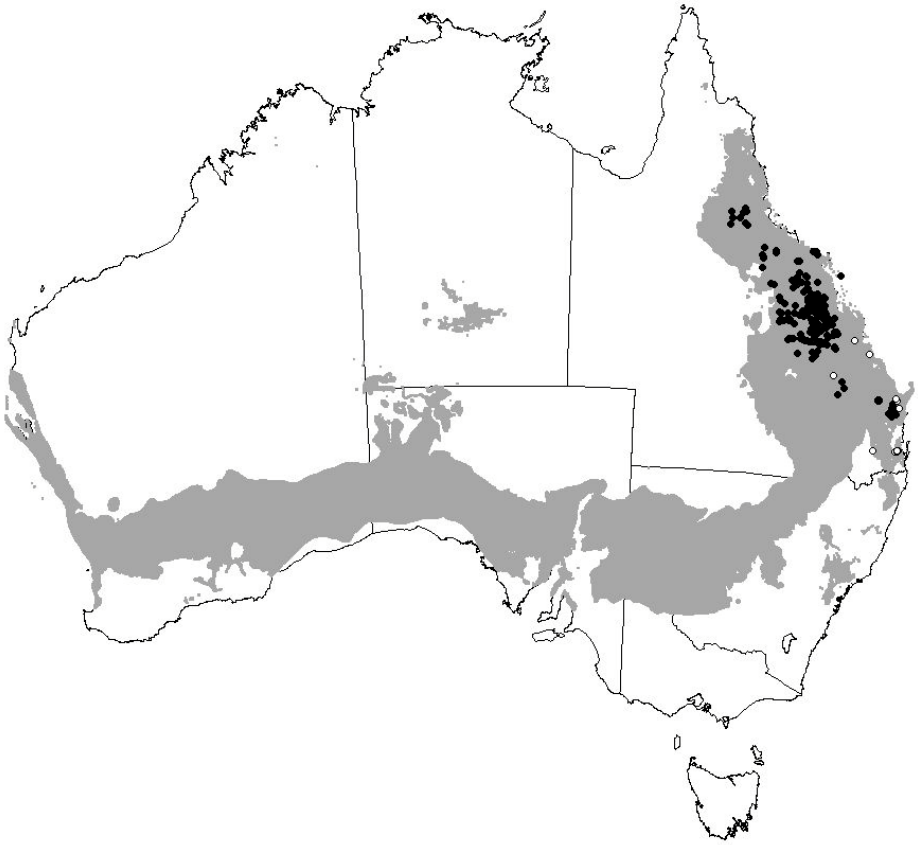

Figure 2.4. Areas suitable for cultivation of *Cassia brewsteri* under irrigated conditions. BIOMAP output showing limitations based on 248 natural (black dots) and 11 cultivated (blue dots) distributions and three Bioclimatic parameters.

Mean maximum temperature of the hottest month (°C)	28.5 - 34.9
Mean minimum temperature of the coldest month (°C)	2.6 - 14.8
Mean annual temperature (°C)	16.3 - 23.4

A seventh variable, absolute minimum temperature, is often used for Australian species that are under consideration for planting in overseas areas where extremes of cold may exceed those in Australian conditions, even where the mean minimum temperature is the same. A number of simulation mapping programs have been developed specifically for forestry programs in Australia, China and other countries where ACIAR conducts forestry programs. These programs use a simplified version of PlantGro (Hackett 1991) to assess the suitability of thousands of locations. In the case of the Australian climate mapping program (GROAUST), a climate profile for the six parameters is matched with a climate database for the six parameters on a 1/8 degree grid of Australia. This program is not currently publicly available (Trevor Booth, CSIRO Forestry and Forest Products, pers. comm.).

Table 2.4. Proposed climatic requirements for *Cassia brewsteri*. Where cultivated sites extended the range of a parameter the estimates from cultivated sites are added in brackets.

Climate parameter	
Mean annual rainfall (mm)	509 - 1616
Rainfall regime (winter, summer, uniform/bimodal)	summer
Dry season length (consecutive months < 40 mm)	0 - 8
Mean maximum temperature of the hottest month (°C)	(28.5) - 29 - 34.9
Mean minimum temperature of the coldest month (°C)	(2.6) - 4.7 - 14.8
Mean annual temperature (°C)	(16.3) - 19.1 - 23.4

Other climate matching programs are available where a climate profile is generated, based on where an organism is known to occur naturally and used to determine other geographic areas where it may potentially spread. A program that has been used in applications such as predicting the potential spread

of weed species is CLIMEX (Sutherst and Maywald 1996) (e.g. McFadyen and Skarratt 1996, Mackey *et al.* 1997). 'Climate' is another program used for weed risk assessment by The Australian Quarantine and Inspection Service (AQIS) when considering entry of exotic plants to Australia. It is based on the principles developed in Bioclim and CLIMEX. The output is plotted on a map of Australia and may be colour-coded to indicate the level of variation from the mean of the climate profile. 'Climate' is based on a Macintosh platform and is distributed as freeware by Agriculture Western Australia (Randall 1999).

Table 2.5. BIOCLIM profile for 248 natural distributions of *Cassia brewsteri.*

Parameter	Mean	S.D.	5%	50%	95%	Max.	Min.
1 Annual mean temperature	21.4	0.8	19.4	21.6	22.6	23.4	19.1
2 Mean diurnal range (mean(monthly max-min))	13.2	1.32	11.2	13.4	14.9	15.1	6.3
3 Isothermality (2/7)	0.53	0.01	0.51	0.53	0.55	0.56	0.43
4 Temperature Seasonality (C of V)	1.48	0.14	1.25	1.48	1.71	1.75	1.06
5 Max temperature of warmest month	32.6	1.42	29.8	32.8	34.7	34.9	29
6 Min temperature of coldest month	7.7	1.44	5.8	7.5	9.9	14.8	4.7
7 Temperature annual range (5-6)	25	2.37	21.7	24.9	28.5	28.9	14.7
8 Mean temperature of wettest quarter	26.1	0.87	24	26.3	27.2	27.7	23.3
9 Mean temperature of driest quarter	16.9	1.09	14.9	16.8	18.8	21.6	14.2
10 Mean temperature of warmest quarter	26.2	0.85	24	26.3	27.3	27.8	23.6
11 Mean temperature of coldest quarter	15.6	0.96	13.9	15.5	17.5	19	12.9
12 Annual precipitation	688	153.4	523	652	1094	1616	509
13 Precipitation of wettest month	121	32.58	96	113	177	324	94
14 Precipitation of driest month	16	6.7	3	17	32	40	0
15 Precipitation seasonality (C of V)	66	11.96	50	64	97	110	47
16 Precipitation of wettest quarter	335	82.5	245	315	468	857	238
17 Precipitation of driest quarter	65	21.12	33	65	120	149	0
18 Precipitation of warmest quarter	330	70.23	249	319	465	747	238
19 Precipitation of coldest quarter	78	23.52	44	73	138	196	30
20 Annual mean radiation	20	0.42	18.8	20.1	20.4	20.4	18.3
21 Highest monthly radiation	25.1	0.3	24.4	25.2	25.4	25.6	23.8
22 Lowest monthly radiation	13.8	0.57	12.2	14	14.7	14.9	11.9
23 Radiation seasonality (C of V)	19	1.19	18	19	23	23	18
24 Radiation of wettest quarter	22.8	0.66	21	22.9	23.4	23.4	20.4
25 Radiation of driest quarter	17.8	0.74	16.4	17.9	18.6	21.2	16
26 Radiation of warmest quarter	23	0.3	22.5	23	23.5	24.2	22.2
27 Radiation of coldest quarter	15.4	0.56	13.9	15.4	16.2	16.5	13.6
28 Annual mean moisture index	0.38	0.11	0.27	0.35	0.68	0.86	0.26
29 Highest monthly moisture index	0.51	0.11	0.38	0.47	0.76	0.97	0.36
30 Lowest monthly moisture index	0.24	0.11	0.14	0.21	0.56	0.73	0.12
31 Moisture index seasonality (C of V)	25	7.67	11	25	44	47	7
32 Mean moisture index of high quarter MI	0.49	0.12	0.36	0.45	0.75	0.96	0.33
33 Mean moisture index of low quarter MI	0.26	0.11	0.15	0.23	0.58	0.75	0.14
34 Mean moisture index of warm quarter MI	0.39	0.09	0.3	0.37	0.62	0.76	0.26
35 Mean moisture index of cold quarter MI	0.39	0.13	0.28	0.35	0.72	0.96	0.27

Table 2.6. BIOCLIM profile for 248 natural and 11 cultivated distributions of *Cassia brewsteri.*

Parameter	Mean	S.D.	5%	50%	95%	Max.	Min.
1 Annual mean temperature	21.4	0.88	19.3	21.5	22.7	23.4	16.3
2 Mean diurnal range (mean(monthly max-min))	13.2	1.38	10.9	13.4	14.9	15.1	6.3
3 Isothermality (2/7)	0.53	0.01	0.51	0.53	0.55	0.56	0.43
4 Temperature Seasonality (C of V)	1.48	0.14	1.24	1.47	1.71	1.75	1.06
5 Max temperature of warmest month	32.5	1.53	29.4	32.7	34.7	34.9	28.5
6 Min temperature of coldest month	7.7	1.48	5.4	7.5	9.9	14.8	2.6
7 Temperature annual range (5-6)	24.8	2.45	21.1	24.8	28.5	28.9	14.7
8 Mean temperature of wettest quarter	26	0.95	23.9	26.2	27.4	27.7	21.7
9 Mean temperature of driest quarter	16.8	1.15	14.7	16.8	19	21.6	11.2
10 Mean temperature of warmest quarter	26.1	0.93	23.9	26.3	27.4	27.8	21.7
11 Mean temperature of coldest quarter	15.5	1.02	13.5	15.6	17.5	19	10
12 Annual precipitation	703	169.35	524	658	1129	1616	509
13 Precipitation of wettest month	123	33.3	96	113	181	324	94
14 Precipitation of driest month	17	7.35	4	16	33	44	0
15 Precipitation seasonality (C of V)	65	12.14	48	63	96	110	46
16 Precipitation of wettest quarter	340	84.34	246	318	474	857	238
17 Precipitation of driest quarter	67	23.62	35	63	122	159	0
18 Precipitation of warmest quarter	335	72.74	250	321	477	747	238
19 Precipitation of coldest quarter	81	27.14	45	74	147	198	30
20 Annual mean radiation	19.9	0.51	18.7	20.1	20.4	20.4	17.9
21 Highest monthly radiation	25.1	0.35	24.1	25.1	25.4	25.6	23.7
22 Lowest monthly radiation	13.7	0.67	12.2	13.9	14.6	14.9	11.3
23 Radiation seasonality (C of V)	20	1.42	18	19	24	25	18
24 Radiation of wettest quarter	22.7	0.74	20.9	22.9	23.4	23.9	19.9
25 Radiation of driest quarter	17.8	0.82	16.2	17.9	18.7	21.2	15.4
26 Radiation of warmest quarter	23	0.33	22.4	23	23.5	24.2	21.9
27 Radiation of coldest quarter	15.3	0.66	13.8	15.5	16.1	16.5	13
28 Annual mean moisture index	0.39	0.12	0.27	0.35	0.71	0.86	0.26
29 Highest monthly moisture index	0.52	0.12	0.38	0.48	0.77	0.97	0.36
30 Lowest monthly moisture index	0.25	0.12	0.14	0.22	0.58	0.73	0.12
31 Moisture index seasonality (C of V)	25	8.03	10	25	44	47	7
32 Mean moisture index of high quarter MI	0.5	0.12	0.36	0.45	0.77	0.96	0.33
33 Mean moisture index of low quarter MI	0.27	0.12	0.15	0.23	0.6	0.75	0.14
34 Mean moisture index of warm quarter MI	0.4	0.1	0.31	0.37	0.64	0.76	0.26
35 Mean moisture index of cold quarter MI	0.4	0.14	0.28	0.35	0.76	0.96	0.27

Table 2.7. Climatic conditions at *Cassia brewsteri* trial sites compared with the climate profile for natural distributions of the species. Bold text indicates climatic parameters outside the climate profile for the species.

Climate parameter	Australia		Thailand			Zimbabwe		Malawi
	Tuan	Wongi	Ratchaburi	Si Sa Ket	Chiang Mai	Makoholi	Kadoma	Makoka
Mean annual rainfall (mm)	1362	1113	964	1586	934	719	812	850-1250
Rainfall regime (winter, summer, uniform/bimodal)	S	S	NA	NA	NA	NA	NA	S
Dry season length (consecutive months < 40 mm)	7	7	NA	NA	NA	NA	NA	6
Mean maximum temperature hottest month (°C)	29.7*	30.1*	NA	NA	NA	NA	NA	NA
Mean minimum temperature coldest month (°C)	9.0*	9.7*	NA	NA	NA	NA	NA	NA
Mean annual temperature (°C)	20.7	20.9	**29.4**	**26.6**	**24.6**	19.2	21.2	NA
Fit to climate profile (no. of parameters matching / no. of parameters available)	6/6	6/6	1/2	1/2	1/2	2/2	2/2	3/3

NA = Not Available, * = calculated using methods of this study.

Table 2.8. Soil conditions at *Cassia brewsteri* trial sites compared to the soil parameters for natural distributions of the species. Bold text indicates soil parameters outside the parameters of the natural distributions sampled.

Soil parameter	Australia		Thailand			Zimbabwe		Malawi
	Tuan	Wongi	Ratchaburi	Si Sa Ket	Chiang Mai	Makoholi	Kadoma	Makoka
Primary profile form	Uniform	Uniform	NA	NA	NA	NA	NA	duplex
Soil colours	Brown / yellow	Yellow / brown	NA	NA	NA	NA	Reddish brown	NA
Texture	Sandy loam / sandy clay loam	Sandy loam / sandy clay loam	NA	NA	NA	Sandy soils / heavy sub-soils	Clay loam	Sandy loam / sandy clay loam
pH (surface)	4.91	4.66	NA	NA	NA	NA	NA	NA
pH (deeper)	5.69	5.01	NA	NA	NA	NA	NA	NA
EC (dS/m) (0-10 cm)	0.173	0.026	NA	NA	NA	NA	NA	NA
EC (dS/m) (40-50 cm)	0.119	**0.014**	NA	NA	NA	NA	NA	NA
Fit to soil profile (no. of parameters matching / no. of parameters available)	7/7	7/7	NA	NA	NA	1/1	2/2	2/2

NA = Not Available.

3. Flowering and fruiting phenology of *Cassia brewsteri*

Abstract

Ripe pods collected from across the range of *Cassia brewsteri* weighed up to 40 g and contained on average 35 seeds comprising 29% of the pod mass. The average seed mass and seed moisture content were 118 mg and 11% respectively. Flowering was observed on seedlings as young as 18 months but this was atypical, flowering and fruiting is thought to commence at 9-11 years. The seasonal variation in timing of flowering events in *C. brewsteri* was analysed using unpublished data from a set of observations made from 1977 to 1996. The mean dates and standard errors for flowering events recorded were as follows: budding / early flowering (9 August ± 12 days); flowering / heavy flowering (13 September ± 10); late flowering (20 October ± 16) and green pods (21 November ± 17). Pods typically ripen in January and February and are retained on the tree for two to 12 months. Flowering and fruiting phenology data were obtained from 60 *Cassia brewsteri* regrowth trees during the 1997-98 season. To assess the effect of water availability, 30 of these trees were irrigated. No significant difference in mean life span of each flowering and fruiting stage was detected between the irrigated and non-irrigated plots. The average length of the flowering and fruiting season for individual trees was 114 ± 13 days, commencing late August. Flowering and fruiting was generally synchronous although several individuals extended the range of the season to five months.

Introduction

Floral structure and pollination

C. brewsteri flowers are borne on a pendulous raceme from 7-50 cm long with 10-50 flowers. The pedicels are 20-30 mm long, bracts and bracteoles persistent until anthesis. The petals are 5-15 mm long and yellow, orange or reddish, the sepals broadly ovate, apex obtuse, c. 6 mm long and pubescent. There are ten stamens, seven fertile with three adaxial staminoides. The filaments are unequal with the shortest adaxial ones 3-4 mm long and the longest abaxial ones c. 15 mm long and sigmoidally curved with a swelling above the mid-point. The anthers are 2 to 3 mm long (Stanley and Ross 1983, Randell and Barlow 1998). The floral description of Randell and Barlow (1998) applies to *C. brewsteri s. lat.,* as the three varieties recognised in their treatment are not distinguished on the basis of floral characteristics.

Pollination biology in *C. brewsteri* is not described in any known references. Randell and Barlow (1998) report that neither the petals or the anthers are UV-reflective but the corolla, when red, should be visible to some bees. In *C. fistula* however, the corolla is highly UV-reflective and the anthers are non-reflective forming an effective dark-spot to pollinating bees. In the *Cassiinae*, pollen is usually shed through short slits or pores in the anthers and released during 'buzz pollination' when bees cause vibration of the flowers (Gottsberger and Silberbauer-Gottsberger 1988).

Retana *et al.* (1994) published a study of carob (*Ceratonia siliqua*) flowering phenology examining the differences between male, female and hermaphroditic cultivars of that species. Part of their methodology has been adapted for the current study although, unlike carob, *Cassia brewsteri* is always hermaphroditic. *C. brewsteri* is semi-deciduous and the timing and extent of leaf shed and new growth are also considered (qualitatively).

Basic phenological information on the timing and length of fruiting season and synchronicity of fruit development is required if *C. brewsteri* is to be grown as a seed crop on a commercial scale. If a breeding program is eventually to be undertaken to develop superior cultivars of the plant, a basic understanding of the breeding system is also essential. The aim of the study was to document the length and synchronicity of the flowering and fruiting season, and the duration and survival rate of each floral stage. The effect of water availability on the flowering-fruiting sequence was also examined.

Materials and methods

Pod and seed morphology

Pods collected from throughout the distribution of the species (Chapter 2) were analysed in terms of pod dimensions (measured with a flexible ruler and a micrometer), seed number and mass of pods and seeds.

Age of trees capable of flowering

Observations were made of trees of known ages in botanic gardens (Rockhampton and Gladstone) and of seedlings cultivated at Central Queensland University, Rockhampton.

Timing, length and synchronicity of flowering and fruiting season

The investigation consisted of (a) an analysis of long-term inter-seasonal variation in flowering and fruiting times using an existing set of data and (b) an intra-seasonal study of phenology on a property west of Dingo in Central Queensland. The effect of water availability on the length of flowering and fruiting season; synchronicity of fruit development and fruit yield was also considered in this part of the study.

Analysis of long term inter-seasonal variation in flowering and fruiting times

Eric Anderson compiled a database of flowering events for *C. brewsteri* and many other species during his travels in Central Queensland since the 1970s. Observations were made in the following categories: Flowering stage, Habitat, Shire, Use, Soils and vegetation community. The data were imported into MS Excel and divided into five sets as follows: (1) Early budding, budding, early flowering, (2) Flowering, heavy flowering, (3) Late flowering, (4) Green fruit and (5) Ripe fruit. Where there was more than one record for a stage within a year (season) the records were averaged to give a single date for that stage for that year. The data were analysed for between-year variation in the timing of each flowering and fruiting stage. The resulting set of data was analysed to determine the mean date, standard error of the mean and range of dates for each stage.

Intra-seasonal phenology study

Site characterisation

'Longdale' is a 10 000 acre grazing property located approximately 10 km west of Dingo (23° 38.006'S 149° 15.444'E). The site was originally woodland dominated by poplar box (*Eucalyptus populnea*) with an understorey of *C. brewsteri* and whitewood (*Atalaya hemiglauca*). The paddock had been cleared several times by ploughing and spraying, most recently in 1993 with a stick rake (15 cm tynes). Many of the woody plants, including *C. brewsteri*, had regrown from their stumps or as root suckers.

In May 1997, two groups of thirty *C. brewsteri* plants were identified. The vicinity of the trees was weeded with a blade brushcutter and applications of herbicides ('Roundup' and 'Access'). Measurements were taken of tree height, number of stems per tree, basal area, and crown diameter (Table 3.1). The physical proportions of the tree on both plots were not significantly different at the beginning of the study suggesting that both plots of trees had the same capacity to bear flowers and fruits.

A solar powered drip irrigation system was constructeded to deliver approximately 3000 L/week to one set of 30 trees with actual delivery monitored with a water meter. Each tree received water through a 'shrubbler' which was adjusted to provide a roughly equal flow rate to each tree, initially 100 L/week. The pump was powered directly from the panel, hence during cloudy periods the water delivery was < 100 L/week and, during intense solar radiation, > 100 L/week.

Table 3.1. 'Longdale' site tree measurements in July 1997 (mean and standard error, n = 30).

Average:	Height (m)	# of stems	Basal area (cm^2)	Crown diameter (m)
Irrigated	1.81 ± 0.04	5.47 ± 0.44	30.99 ± 2.92	1.73 ± 0.07
Control	1.75 ± 0.04	4.93 ± 0.39	27.43 ± 2.95	1.71 ± 0.05
Both plots	1.78 ± 0.03	5.20 ± 0.29	29.21 ± 2.05	1.72 ± 0.04

Between 15 July and 30 October 1998, 32,000 L of water was dispensed to the irrigated plot i.e. an average 2070 L/week or 69 L/tree/week. However, as the system was subject to occasional leaks caused by stock damage not all of this volume was actually delivered to all of the trees. Indeed, due to mechanical problems with the irrigation system there was no difference in the amount of water available in November, shortly after fruit set. This coincided with a hot, dry period that adversely affected both plots.

Rainfall records were obtained from farm diaries dating back to 1930, these were compared with figures purchased from the Bureau of Meteorology and based on the weather station at Dingo Post Office (approximately 10 km east of the site). The rainfall at 'Longdale' is low and variable, however the pattern is consistently summer rainfall with over half the annual average of 690 mm falling in the three months December-January. Annual rainfall ranges from 238 mm to 1351 mm with a median of 663 mm. The average number of rain days is 46.6 ranging from 23 to 83 (Bureau of Meteorology data 1896-1998). The average rainfall recorded at the Bureau of Meteorology weather station at Dingo was similar in level and pattern to the rainfall records of the farm diaries at 'Longdale'. The rainfall pattern during the current study (1997) featured a lower than average winter rainfall with a higher than average December rainfall.

Flowering status

A scale of floral development was designed to represent each stage in the flowering and fruiting phenology of *C. brewsteri* (Table 3.2). The data were entered into a spreadsheet and a weighted mean stage for each inflorescence calculated for each week. The duration of each stage was calculated through regression analysis of the data taken on the mean developmental stage of inflorescences (after Retana *et al.* 1994). The average number of inflorescences per tree was estimated by counting the inflorescences of five representative trees in each plot.

Table 3.2. Proposed flowering and fruiting stages of *Cassia brewsteri*

Stage	Description
1	Inflorescence buds green, < 1 mm. Individual flowers distinguishable but closed, not clearly separated or on stalks.
2	Inflorescence buds red or green. Individual flowers 1 mm or larger, still closed but sepals distinguishable. Stalks visible, 1-20 mm long, longer towards base of inflorescence.
3	Sepals open but petals still closed Inflorescence greenish. Inside flower pistils and anthers clearly distinguishable, but not clearly developed.
4	Styles and stigmas green, but not completely developed. Anthers completely developed but filaments still curled up. Petals partially open.
5	Pistils and stamens completely developed. 3 main Anthers undehisced. 4 anthers may be dehiscing. Petals fully open, stigmas yellowish.
6	3 main anthers dehiscing or dehisced, 7 reduced anthers withering. Petals withering, tip of stigma may be black.
7	Beginning of fruit set, pods distinguishable, green, > 20 mm, < 50 mm (clearly longer than anther filaments, usually straightened).
8	Pods half-sized, green (8-15 cm).
9	Pods full-sized, green (15 cm +, straightened and widened).
10	Pods ripe, brown and hard.

Flowering status results were recorded weekly from the first sign of flowering for each of the thirty trees in the two irrigation treatments. Five inflorescences were tagged using aluminium surveying tags and copper wire on each of two branches on each tree. The tagged inflorescences were located approximately on the north and south side of the tree. Each week the survival rate of whole

inflorescences was recorded and aborted inflorescences replaced by tagging new ones, initially back to five and later back to three on each branch.

Results and discussion

Pod and seed morphology

Pod length, width and thickness were all extremely variable both across the range of *C. brewsteri* and, to a lesser extent, within each region where samples were collected. Pod length ranged from 10 to 55 cm (n = 148), width from 1.17 cm to 2.65 cm (n = 108) and thickness from 0.80 cm to 1.70 cm (n = 108). The compression or degree of flattening of the pods, expressed as thickness/width, ranged from 0.46 to 0.96 (i.e. from flattened to almost circular in cross section). The fresh mass of (ripe) pods ranged up to 40.3 g.

The pod shape and surface morphology was variable with three main types being observed. These may be described as flaking (surface) and flattened (shape) (FF), smooth and flattened (SF) and smooth and round (SR). The fourth possible morphology in this classification, flaking and round (FR) was not observed. To a certain extent, the flaking of the pod surface may be related to weathering and the time the pod has remained on the tree after ripening. However, many SF pods were observed to retain their smooth surface for long periods on the tree, and collected SF pods also maintained their surface texture. Microscopic analysis revealed very few or no surface hairs in *C. brewsteri* pods compared to *C. tomentella* pods, a diagnostic character used in identification keys for the genus in Australia (e.g. Symon 1966).

By far the most commonly observed pod type was FF, with SF the next most common. In these pods the seed compartments are visible as transverse ridges across the pods with the longitudinal ridges clearly protruded. In SR pods, this compartmentalisation is not clearly visible externally. The only pod morphology that was observed in *C. tomentella* was SR. The SR morphology was observed in only one case in *C. brewsteri* (site 17) and may represent a hybrid with an intermediate pod form (Chapter 1).

The proportion of seed calculated from fresh weights ranged from 10.8% to 44.7% of the pod mass (mean 29.3%, n = 146 pods). For a smaller set of 18 pods where the three components of the fruit were separated, the seed comprised a mean of 33.0%, the pith 34.1% and the pod wall 32.8%. Although the proportions vary considerably, the average pod can be considered to consist of approximately equal parts of the three types of tissue.

Seed number per pod ranged from 7 to 60 (mean 35, n = 127 pods). Seed size, shape and colour were extremely variable (Chapter 6, Fig. 6.2). The ripe seed mass ranged from 53 mg to 205 mg (mean 118 mg, n = 127 pods). For seeds which were ground for further tests the seed moisture ranged from 7.5% to 14.1% (mean 11.3%, n = 46 seed samples, fresh weight 4 g). There was only a slight positive correlation between ripe seed mass and seed moisture (linear $r^2 = 0.21$, n = 46). It can be concluded that larger seeds contained more endosperm (or embryo) and were not simply less desiccated.

Age of trees capable of flowering

The plant age at which flowering commences is not well documented. Ryan and Bell (1989) observe that no flowering had occurred by age 31 months on four plots of 36 trees grown in Wongi and Tuan State Forests, southeast Queensland. Williams (1979) notes that young specimens are capable of flowering at a height of around one metre although the age is uncertain, as these specimens may have regrown as root suckers.

Of over one hundred *C. brewsteri* seedlings cultivated during the course of this study only one flowered, at age 18 months under outdoor conditions with daily watering. The inflorescence developed to maturity but did not bear fruit. Flowering at this age should be considered an anomalous event.

Toondoon Botanic Gardens in Gladstone features four well-established *brewsteri* about 8-10 m tall. When the trees were planted from pots in 1987 they were probably one year old, i.e. 12 years old in 1998. The trees have flowered lightly from the age of nine and borne few pods (Gladstone Parks technical staff, pers. comm.).

Several trees in the ACIAR trial in Wongi and Tuan State Forests have reached 6.5 m in 12 years and first flowered from year 11 or earlier (Cliff Raddatz, DPI Forestry, pers. comm.). Another set of cultivated trees at Gladstone's Toondoon Botanic Gardens has reached eight to 10 m in 11 years and first flowered at year nine. A tree in the Kershaw gardens at Rockhampton yielded fruit at an estimated age of six to eight years.

Inter-seasonal timing, length and synchronicity of flowering and fruiting

The long term observations of *C. brewsteri* covered the period 1977 to 1996 and a geographical region bounded by latitudes 20.5° to 26.0° S and longitudes 147° to 152.5° E. The range, mean dates and standard error of the mean (in days) for flowering events were determined (Table 3.3).

Table 3.3. *Cassia brewsteri* flowering time over a 20 year period (1977-1996).

	budding, early flowering	flowering, heavy flowering	late flowering	green fruit	ripe fruit
Mean (date)	9 August	11 September	13 October	29 November	30 March
Standard error (days)	12	10	16	17	28
Earliest record	9 June	5 July	1 July	12 September	24 January
Latest record	27 September	31 October	2 December	26 February	1 June
Number of records	21	36	13	13	4
Number of years	9	16	8	8	3
Time period	1979 to 1989	1977 to 1996	1979 to 1994	1978 to 1991	1984 to 1987

The length of time from green fruit to ripe fruit was calculated as 121 days. This estimate is probably an overestimate due to two factors. Firstly the number of records for the final stage was small (four in only three years) and secondly the fruit may have been ripe for long periods (several months) before the record was made. From 1997 to 2000, fruit typically ripened in January to February and was retained on the tree for two to 12 months.

Intra-seasonal timing, length and synchronicity of flowering and fruiting

Dormancy patterns

Three patterns of dormancy were observed. In some cases full leaf shed was followed by a period of dormancy after which vegetative and reproductive growth commenced at more or less the same time. In other cases partial leaf shed was followed by reproductive growth. In rare cases, no leaf shed occurs and flowers may or may not be set. Seedlings (c. 50) maintained in a glasshouse commonly shed all their leaves for a period of several weeks commencing in August 1999. These seedlings were watered daily which suggests that the onset of dormancy may be related to photoperiod as well as water availability.

Deciduous tree species are uncommon in the Australia flora, in northern Australia two examples are *Terminalia ferdinandiana* and *Planchonia careya*. In tropical forests, leaf-fall generally coincides with the dry season and leaf-flush with the start of the wet season although flushing may commence prior to rain (Myers *et al.* 1998). *C. brewsteri* on the control plot shed their leaves more or less synchronously and remained dormant for longer than the irrigated plot which suggests that the control plot was drier and this was a cue for leaf-shed. A simple interpretation like this must be treated with caution however, as the quantitative data (not shown) was not statistically robust and other factors may also be important in the timing of leaf phenological events. Myers *et al.* (1989) experimented with irrigating both deciduous and non-deciduous trees in the northern tropics of Australia to determine the effect of

soil moisture on leaf phenology. Soil moisture variation had a minor effect on the leaf phenology of one deciduous species and no effect on another. The authors concluded that stem water status and vapour pressure deficit may be important climatic cues in leaf-fall and leaf-flush in tropical deciduous trees. The leaf phenology of *Cassia brewsteri* requires more investigation if it is to be domesticated as an orchard species. Since it is likely to be an outcrossing species (see introduction and discussion below), an orchard would need to be managed in such a way as to promote synchronous leaf-flush and flowering to maximise the availability of pollen at the beginning of inflorescence.

Life span of each reproductive stage

An approximation of time to ripening can be made based on the starting time of flowering, the earliest time of fruit set and the latest time of fruit ripening for each whole plot (Table 3.4). The approximate fruiting season for *C. brewsteri* at Dingo in 1997/98 was 5 months, commencing late August / early September.

Table 3.4. Range of the flowering and fruiting season for *Cassia brewsteri* at **'**Longdale' in 1997/98.

Plot	Start of flowering to fruit set (days)	Fruit set to ripening (days)	Total (days)
Irrigated	42	113	155
Control	56	92	148
Average	49	103	152

Records of annual variation in *C. brewsteri* flowering time have been published based on herbarium collections. Forster (1991) reports flowering records of September to November and fruiting records of January to April for *C. brewsteri*. In a report of seed collections for 112 Queensland tree species, Searle (1989) notes seeding times for *C. brewsteri* of January and February, but no flowering times are recorded.

The average length of the flowering and fruiting season for individual trees was 114 ± 13 days. No significant difference in mean life span of each flowering and fruiting stage was detected between the two irrigation treatments (Table 3.5). Flowering and fruit set on the irrigated plot commenced earlier and was less synchronous than on the control plot, possibly because of variability in water supply to the individual trees (Fig. 3.3).

Table 3.5. Mean life spans of *Cassia brewsteri* flowering and fruiting stages in 1997 (n = 30).

Stage	Control plot mean (days)	n	Irrigated plot mean (days)	n
1	12.1 ± 0.3	13	9.3 ± 0.4	25
2	4.1 ± 0.5	28	4.8 ± 0.4	53
3	2.8 ± 0.2	29	2.8 ± 0.1	51
4	2.7 ± 0.2	28	2.6 ± 0.1	51
5	3.7 ± 0.4	26	3.9 ± 0.4	48
6	4.7 ± 1.3	3	6.5 ± 2.1	3
7	22.3 ± 5.4	4	11.5 ± 4.5	2
8	11.3	1	26.0 ± 5.4	6
9	56.0	1	41.8 ± 3.7	6
1 to 6	30.1 ± 2.9		29.9 ± 3.6	
7 to 10	89.58 ± 5.4		79.3 ± 13.5	
1 to 10	120 ± 8.2		109 ± 17	

The number of inflorescences per tree was 70 ± 10.3 on the irrigated plot and 14 ± 3.4 on the control plot (mean and standard error of 5 trees at a mean stage of 5.7). All 30 trees on the irrigated plot commenced flowering, compared to only 24 on the control plot. Ten trees bore fruit on the irrigated plot compared to only three on the control plot (Fig. 3.1). The decline in progression from stage 6 to stage 7 on both plots may be due to the dry conditions experienced at the time (November). At this time, both plots experienced low inflorescence retention (Fig. 3.2). The progression to further floral stages, in terms of trees per plot, declined slowly on the control plot in contrast to the irrigated plot

where all trees progressed to stage six but only a few continued beyond this. These trends point to the conclusions that water availability limited the progression through floral developmental stages and that the higher developmental stages were more dependent on this resource.

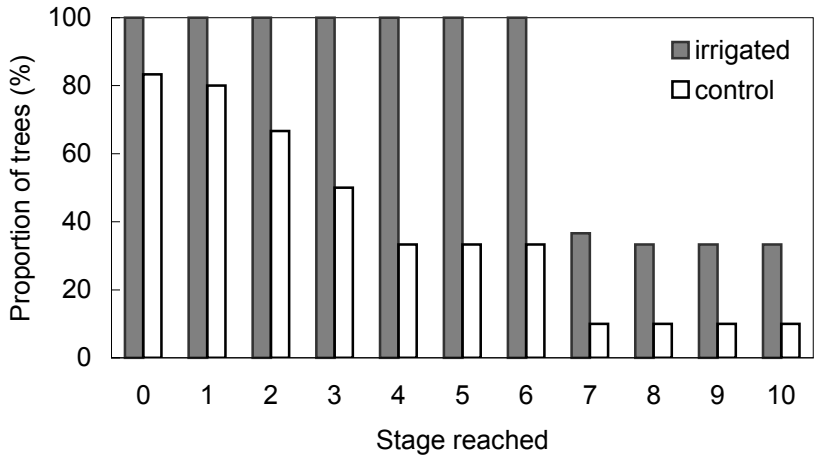

Figure 3.1. The proportion of *Cassia brewsteri* trees with at least some flowers progressing to each stage on irrigated and non-irrigated plots.

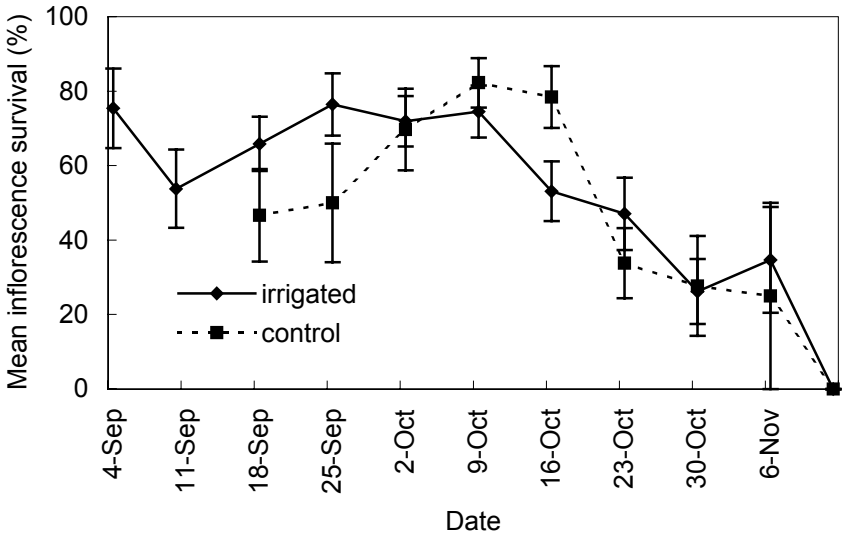

Figure 3.2. Proportion of *Cassia brewsteri* inflorescences surviving one week or more over the 1997 season (mean and standard error). Diamonds represent the irrigated plot, squares the non-irrigated plot.

The average survival of tagged inflorescences, defined as the proportion surviving one week or more, on the control plot over the season was 53.0 ± 3.1 % (mean and standard error of 24 trees) (Fig. 3.2). On the irrigated plot, survival of tagged inflorescences was not significantly higher, at 57.1 ± 1.7 % (mean and standard error of 30 trees). The rate of inflorescence abortion increased toward the end of the season, again there were no clear differences between the irrigated and control trees. Inflorescence survival data (Fig. 3.2) were replotted with reference to floral stage (Fig. 3.3). Survival rate of whole inflorescences tended to decrease with increased floral maturity.

Of the 4100 individual flowers monitored throughout the study, only 38 developed to form mature pods. This equates to 1.0% or a fruit to flower ratio (Fr:Fl) of 0.01. Bawa and Buckley (1989) report considerable variation of Fr:Fl within the Caesalpiniaceae, e.g. 0.01 for *Caesalpinia eriostachys*, and 0.10 for *Bauhinia ungulata*, both trees or tall shrubs. *Senna pallida* (an herbaceous shrub) and

S. bicapsularis (a forest tree) had Fr:Fl ratios of 0.60 and 0.09 respectively. Of these four species, only *S. pallida* is inbreeding, although it does predominantly outcross. Bawa and Buckley's (1989) comparison supported earlier work indicating that there is a strong correlation between Fr:Fl ratio and the degree of outcrossing in many species. The low estimated Fr:Fl ratio of *Cassia brewsteri* suggests that, like many *Cassia* species, it is outcrossing.

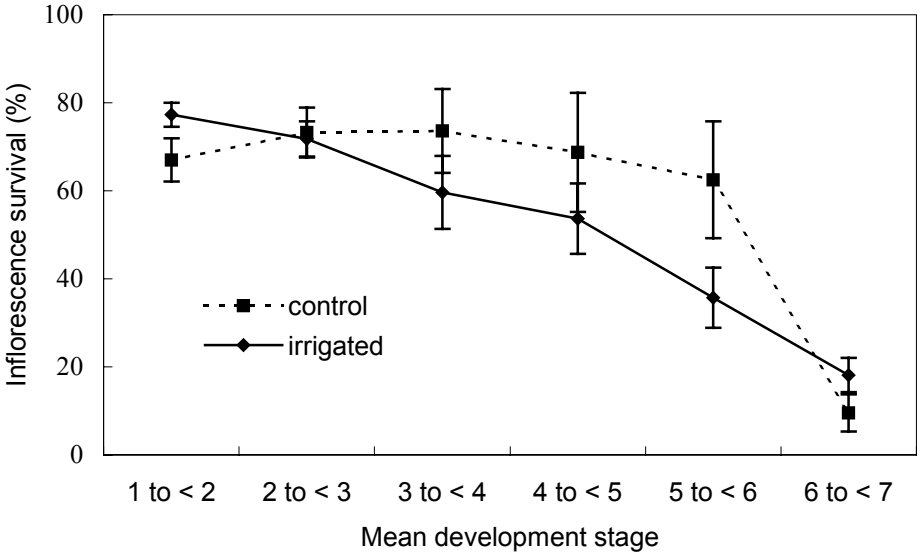

Figure 3.3. Proportion of *Cassia brewsteri* inflorescences surviving one week or more at each developmental stage (mean and standard error).

The low fruit yields recorded for *C. brewsteri* in this investigation are, on the surface, not encouraging for the prospects of developing a commercial seed crop. However, the data was collected in order to analyse flowering and fruiting times and was not intended to be the basis of yield models. Although one of the plots was irrigated for part of the season the soil moisture overall was very low compared to a commercial orchard. In addition to this, the plants themselves were wild, unimproved stock. Seed quantity is the best criterion for distinguishing a domesticated plant from its wild relatives. Plitmann and Kislev (1989) describe several other phenological changes that have been brought about in the domestication of leguminous species for food production including changes in the timing and duration of flowering, loss of photoperiodic sensitivity, loss of dispersal mechanisms and higher inbreeding.

While most of the legumes grown as crops today have been bred for thousands of years to induce the changes required for a modern commercial crop there are many examples of more rapid domestication. The only Australian plant to be developed as a commercial fruit crop to date is the macadamia nut (*Macadamia integrifolia*). The main cultivars of this species now grown in Australia were developed in Hawaii where the industry was first established on a commercial basis in the. Moncur *et al.* (1985) report a fruit to flower ratio in macadamia of around 3%, i.e. three times that reported here for *C. brewsteri*, and note that yield increases due to irrigation of macadamia have been obtained for many years. Further investigation of the effect of water stress at different phases of development in *C. brewsteri* is required to develop an understanding of these processes and how they can be manipulated to optimise seed yield.

4. Propagation of *Cassia brewsteri*

Abstract

Propagation of *Cassia brewsteri* by seed germination, stump planting and cuttings was investigated. Rapid emergence from seed required pre-treatment of the seed. Mechanical scarification was the most effective producing 82 ± 5% emergence by day 25, followed by acid scarification (72 ± 4%). Immersion in hot water for 5 minutes (25 ± 6%) gave a similar result to no pre-treatment (18 ± 5%). Immersion in a water bath with an agitator for 48 hr at 40 °C produced very poor results (7 ± 5%). Emergence of ineffectively treated seed continued for at least 12 months. Propagation by stump planting was successful in only one of five attempts. No successful rooted cuttings were produced from new growth and one to two year old growth obtained in Autumn, Spring and Summer.

Introduction

The advantage of seedlings in cultivation is that they are cheap and easy to obtain, while their disadvantage lies in their genetic variability. Germination of hard-coated seeds such as those of *Cassia* species has been studied extensively and many pre-treatments are recommended to allow the seed to soak up water and hence germinate. The advantage of vegetative propagation lies in the fact that trees selected or bred for particular traits can be multiplied to produce an entire orchard of trees with the desired characteristics e.g. high seed yield and high quality seed. Vegetative propagation techniques include rooted cuttings, aerial layers, grafting onto the rootstock of other seedlings and tissue culture techniques.

Wrigley and Fagg (1979) recommend scarification of the seed to promote germination of *C. brewsteri* and the other native *Cassia* species, but a specific method was not given. Leiper and Thompson (1991) recommend scarification of *C. marksiana* seed (no method specified), and state that treatment with boiling water gave a marginal germination response (no data presented). The Australian Tree Seed Centre (ATSC 1998) recommend pre-treatment of *C. brewsteri* seed by manual nicking/scarification, followed by pouring on boiling water (100 °C) and soaking until cool. They report a germination rate of 64 seeds per 10 g (at a typical mass of 0.1 g per seed this equates to 100 seeds and thus 64% germination). The ATSC germination test involved were one sample of 10 g of germinated seed (collected 5 Feb 1986 from north of Marlborough) on a substrate of vermiculite at 25 °C with eight to 12 hr of light per 24 hr cycle. The germinates were counted firstly on day five and lastly on day 12.

Germination studies have been reported for *Cassia fistula* where the most effective pre-treatment is acid scarification. Sheikh (1980) reported a 68% germination rate (135 out of 200 seeds after 24 days) for seeds soaked in H_2SO_4 for 30 min. In contrast, soaking seeds in boiling water for 30 min or keeping them in cow dung for two weeks proved ineffective in promoting germination. Ahmad and Khattak (1980) reported a germination rate of 90% after 20 days following soaking in H_2SO_4 for 15 and 20 min. Soaking in H_2SO_4 for 1 hr then hot water for 24 hr yielded 76% germination.

The only methods of vegetative propagation previously recommended for *C. brewsteri* are aerial layering or grafting onto young seedlings (Elliot and Jones 1982). No special methods are described for *C. brewsteri* and no quantitative data have been reported. Air layers have been successfully propagated from *Cassia fistula*, *C. javanica* var. *indochinensis* (*C. nodosa*) and *Senna siamea* (*C. siamea*) (Misra and Jaiswal 1994 and 1995). Ten year old trees of *C. fistula* and *C. nodosa* and six year old *S. siamea* were used for layering trials with a range of concentrations of indole butyric acid (IBA) from zero to 25,000 ppm. The IBA was applied in a lanolin paste to the upper side of girdled lateral shoots (two year old shoots of 10 year old *Cassia* spp. and six year old *S. siamea*). Sphagnum moss was used as a rooting medium. Layering was done in July (summer in India) and the layers severed in October (autumn). Maximum rooting occurred at 25,000 ppm for *C. fistula*, 10,000 ppm for *C. nodosa* and 15,000 ppm for *S. siamea*. The optimal concentration of IBA for seven year-old trees of *C. siamea* was also 15,000 ppm (in the range 0-20,000 ppm) (Misra and Jaiswal 1994 and 1995).

Jolin and Torquebiau (1992) describe a technique traditionally used in Costa Rica to produce live fences from large cuttings of several tree species including *Cassia grandis*. The method involves selecting crown suckers of about 15 cm in diameter from well-developed trees in March (spring). The shoots are trimmed to 2.5 m and laid horizontally in the shade for a week then stacked vertically for three weeks. Four weeks after cutting the shoots are planted to a depth of 50 cm. In some species, more than 80% of shoots planted in this way develop into mature trees.

Gharyal and Maheshwari (1990) obtained stem and petiole explants from mature *Cassia fistula* and *Senna siamea* trees. These formed callus and differentiated shoot-buds and shoots on B5 medium supplemented with either 0.5 mg/L indole acetic acid (IAA) + 1 mg/L BA or 2 mg/L naphthalene acetic acid (NAA) + 0.5 mg/L BA. Plantlets of *C. fistula* were obtained under conditions conducive to rooting, i.e. basal medium alone or supplemented with 0.1 mg/L IAA. It is likely that *C. brewsteri* could be similarly cultured.

In the current study, the propagation of *C. brewsteri* has been considered in terms of sexual and asexual systems, involving seed germination studies and propagation by shoot and root cuttings.

Materials and methods

Effects of pre-treatment on emergence of Cassia brewsteri seed

Seeds of *C. brewsteri* were obtained through a commercial seed supplier, Queensland Tree Seeds (QTS), sourced from an unspecified locality in Central Queensland. Five different treatments were imposed on seed prior to imbibition:

1. Mechanical scarification (with a scarifying machine operated by QTS)
2. Acid scarification by immersion in 90% (v/v) sulphuric acid for 20 min (five volumes of acid for one volume of seed) followed by immersion in a water bath with an agitator for 48 hr at 40 °C
3. Boiling water poured over the seed and allowed to stand for 5 min (five volumes of water to one volume of seed)
4. No pre-treatment
5. Immersion in water bath with an agitator for 48 hr at 40 °C.

Following pre-treatments, seeds were arranged in five rows of twelve seeds on each of five separate trays. Each tray contained one replicate of each pre-treatment (i.e. a randomised block design). The trays contained one part sand to one part perlite to one part peat, and the seeds were covered with 0.5 cm of the same medium. The trays were located in a glasshouse under an automatic watering system (sprinkler activated twice per day). The experiment was conducted in Rockhampton, Central Queensland and day zero was 3rd October, 1997. For practical purposes, emergence, defined as the stage at which any part of the plant emerged above the medium, was recorded rather than actual germination in a controlled environment. This allowed the continuation of the study over a 12 month period to assess the germination dynamics of the soil-borne seedbank of wild stock in addition to the short term information required to produce recommendations for nursery practice. Comparison of results was done by ANOVA and Tukey's Studentized Range (HSD) test.

Propagation of *Cassia brewsteri* by cuttings

Cutting propagation was attempted using three types of shoot cuttings collected in three seasons. New growth and one to two year old growth were collected in autumn, spring and summer while material older than two years was collected in autumn only. The main hormonal treatment used was a commercial preparation, 'Multicrop Take Root' with the active constituents 0.5 g/kg IBA and 0.5 g/kg NAA (i.e. 0.05% IBA, 0.05% NAA). One set of 12 one to two year old shoots was treated with an aqueous solution of 0.3% IAA and 0.3% NAA (dissolved first in a small volume of ethanol).

The cuttings were placed in a medium comprised of one part sterilised sand and one part fine grade perlite. This medium was kept at 22-25 °C in a bottom-heated tray in a misthouse. Results were recorded every one to two weeks for a period of three months. The number of cuttings with shoot

growth was counted and several cuttings from each treatment were carefully extracted from the medium and checked for signs of root initiation.

Propagation of *Cassia brewsteri* by stump planting

Five large sections of roots taken from a blade-ploughed paddock in March and April 1997 were planted in mounded sandy soil. The sections were from 0.5 to 1.5 m long and four to 12 cm in diameter with some roots and shoots on the main section. Water was applied several times per week.

Results

Effects of pre-treatment on emergence of *Cassia brewsteri* seed

Tukey's multiple comparisons test ($\alpha = 0.05$) revealed that at day 25 the two most effective pre-treatments, mechanical scarification (82 ± 5% emergence) and acid scarification (72 ± 4%) were not significantly different from each other (Fig. 4.1). They were, however, significantly different from the control treatment (18 ± 5 %), immersion in hot water for 5 minutes (25 ± 6%) and immersion in a water bath with an agitator for 48 hr at 40 °C (7 ± 5%). There was no significant difference within these three groups.

Emergence of ineffectively treated seed continued for at least 12 months. Seeds which had been ineffectively pre-treated continued to emerge at a relatively constant rate (Fig. 4.2). By day 365 there were no significant differences in the cumulative emergence of the five groups (P = 0.1229).

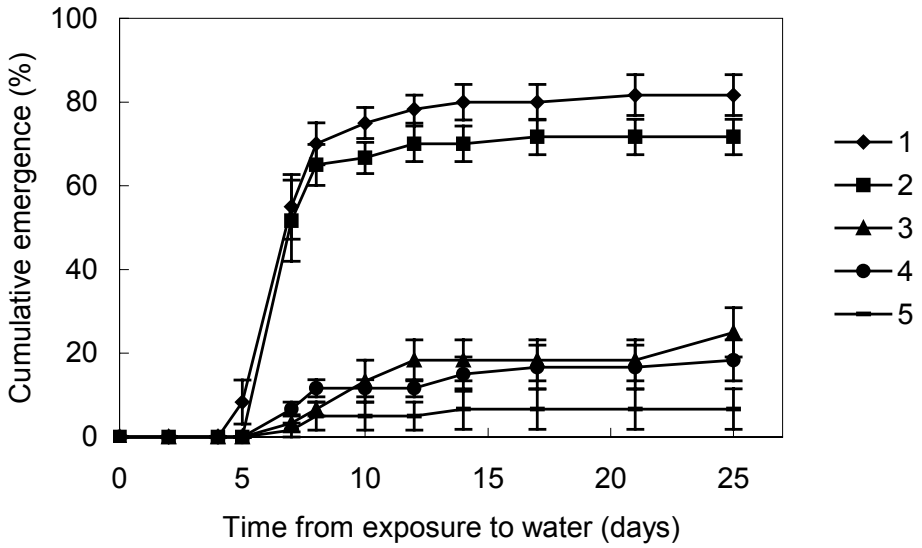

Figure 4.1. Cumulative emergence rate of *Cassia brewsteri* seeds with different pre-treatments over 25 days. Each point represents the mean ± standard error of five replicates of 12 seeds each. (1) Mechanical scarification; (2) acid scarification and 48 hr soaking in 40 °C water; (3) 5 min soaking in boiling water (4) no pre-treatment; (5) 48 hr soaking in 40 °C water.

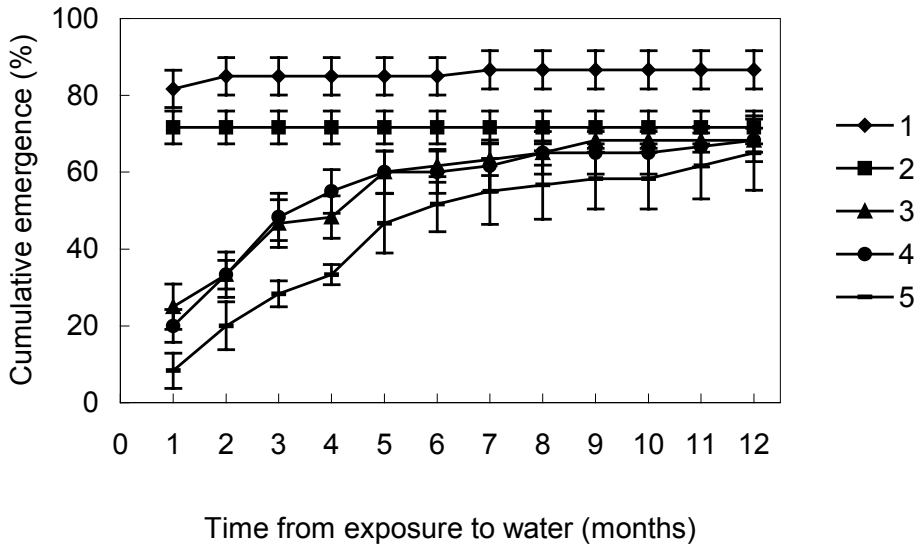

Figure 4.2. Cumulative emergence rate of *Cassia brewsteri* seeds with different pre-treatments over 12 months. Each point represents the mean ± standard error of five replicates of 12 seeds each. (1) Mechanical scarification; (2) acid scarification and 48 hr soaking in 40 °C water; (3) 5 min soaking in boiling water (4) no pre-treatment; (5) 48 hr soaking in 40 °C water.

Propagation of *Cassia brewsteri* by cuttings

Shoot meristem initiation occurred in most cases (Table 4.1), and reached a maximum for all cutting types at days 41, 14 and 14 in trials one, two and three, respectively. Subsequently, the shoots gradually died off over several weeks. None of the cuttings produced roots. Curiously, in some one to two year old growth cuttings, floral growth was initiated and some flowers developed to anthesis.

Table 4.1. Shoot formation in *Cassia brewsteri* cuttings.

Type of cutting (age)	Season	Mean % of shoot-producing cuttings	Standard error	Trial number
< 1 yr (c. 7 months)	Autumn	35	7	1
< 1 yr (c. 2 months)	Spring	0**	0	2
< 1 yr (c. 4 months)	Summer	75	17	3
1 to 2 yr	Autumn	78	15	1
1 to 2 yr	Autumn	65	15	1*
1 to 2 yr	Spring	80	6	2
1 to 2 yr	Summer	28	12	3
2 yr +	Autumn	92	2	1

* = cuttings treated with 0.3 % IAA, 0.3 % NAA all others 0.05% IBA, 0.05% NAA
** = misthouse offline for 5 days with outside temperature at c. 35°C (c. 27 October)
Trial 1: commenced 02/05/97, 5 replicates, 20 cuttings in each treatment
Trial 2: commenced 03/10/97, 5 replicates, 12 cuttings in each treatment
Trial 3: commenced 06/12/97, 3 replicates, 12 cuttings in each treatment

Propagation of *Cassia brewsteri* by stump planting

Only one of the five root sections produced new roots and shoots. This successful explant was a relatively small section (< 50 cm long × < 5 cm diameter).

Discussion

Seedling emergence

Mechanical scarification of seed was the most effective pre-treatment. This method is also simple to implement, avoiding the difficulties of handling concentrated acid and maintaining hot water immersion, and is therefore recommended for nursery use.

The emergence of ineffectively treated seed continued for at least 12 months under constant conditions of high moisture. This response is suited to the pronounced summer rainfall pattern inherent in the environment of the natural range of the species. Seeds would not be recruited to the soil seedbank until the end of the wet season (Chapter 3), and hence survival of seedlings would be unlikely if they germinated immediately. Acid treatment such as partial digestion by animals like bandicoots or mechanical scarification by soil particles during heavy rainfall of the following wet season could serve to breach the testa, priming the seeds for germination in subsequent wet seasons. Bandicoots inhabit at least part of the plant's range and have been observed stripping seed from the pods (Jim Niall, QTS, pers. comm.). Herbivory could also provide a dispersal mechanism for the plant.

Once the testa is breached and external water is available, the galactomannan-based endosperm will hydrate, absorbing several times its own weight of water (Chapter 6). The endosperm will therefore serve the developing embryo as both a water reservoir and a source of carbohydrate. To assess the 'water reservoir' role, it would be instructive to monitor germination of scarified *C. brewsteri* seed under wetting and drying cycles, relative to a similar sized seed without galactomannan reserves.

Natural propagation

The natural means of propagation of *C. brewsteri* has not been reported. Following the widespread clearing of land for grazing, a large proportion of the current population of *C. brewsteri* is made up of trees that have grown from suckers. These develop from either the roots or trunks of trees where they have been knocked down by bulldozer, chain or blade plough. A healthy mature tree may also produce root suckers, particularly on disturbance of the root system. Natural stands of *C. brewsteri* also frequently occur as clusters, indicative of spread by root suckering from an original seed source. Indeed, despite the heavy seed sets observed in the species, no seedling recruitment was observed on natural stands (Chapter 2), all regeneration was ascribed to root suckering. It is concluded that seed germination and establishment under natural conditions occurs relatively rarely, probably in episodic above-average rainfall years. The depletion of viable seed recruitment to the soil seed reserve due to predation by bruchids (Chapter 5) may impact on future seedling establishment rates.

Commercial propagation

The failure to produce rooted cuttings may be due to inherent difficulties with the species or inappropriate methods used to strike cuttings. If the latter is the case, the most likely problem is with the hormonal treatment used. Other treatments using different active ingredients and/or different concentrations and/or a different mode of application such as a gel may yield better results. Root/stump planting of *C. brewsteri* may be worth further consideration. However, stump planting does not offer multiplication potential, and sourcing root cuttings is more difficult than shoot cuttings.

If the species is not amenable to clonal propagation by cuttings, the alternative of aerial layers and grafting onto young seedlings may be the most effective way of producing trees for orchard establishment. Grafting selected varieties onto young seedlings has been used for carob (*Ceratonia siliqua*) for centuries in the Mediterranean region. Grafting is still the most common propagation technique for carob and represents about 10% of orchard establishment costs (Race *et al.* 1999). Growers have focussed on selective multiplication of female plants with the aim of producing higher fruit yields. Thomas and Mehta (1983) have explored the use of *in vitro* methods for rapid propagation of carob. However, there have been no subsequent reports of the application of these methods on a commercial scale.

5. Impact of the Peanut Bruchid (*Caryedon serratus*) on *Cassia brewsteri* seed yields

Abstract

The distribution of *Caryedon serratus,* the peanut (groundnut) bruchid, on two Australian native plants *Cassia brewsteri* and *C. tomentella* was documented over two years. *Caryedon serratus* was observed across the central and northern parts of the range of *Cassia brewsteri* (latitudes 19.258 - 24.140° S) and at least part of the range of *C. tomentella* (as far as 24.427° S). Seed loss to *Caryedon serratus* in these species, assessed across all collection sites, was 40 ± 8.0%. Where the bruchid was detected at a given site, 72 ± 8.6% of pods on 71 ± 8.5% of trees were affected. Additional distribution points and other potential host species from previous *Caryedon serratus* collections in the Australian National Insect Collection (ANIC) are reported. The combined data were used to produce a predicted potential range for the bruchid across the dry tropics of Australia. No reports of migration to cultivated or stored peanut (*Arachis hypogaea*) in Australia were located. Further investigation of the potential impact of this bruchid on the Australian peanut industry is recommended. A potentially beneficial aspect of *C. serratus* establishment may be the biological control of *Acacia nilotica* in Australia.

Introduction

Caryedon serratus Olivier (*Caryedon gonagra* Fabricius) is a bruchid (Coleoptera) that develops in the seeds of leguminous plants. Reviews in the mid 1990s recognised eight confirmed host species all of which belong to the Caesalpiniaceae with the exception of peanut (*Arachis hypogaea* Fabaceae) (Pierre and Huignard 1990, Nilsson and Johnson 1992, Delobel 1995). *C. serratus* is thought to have shifted to peanut from trees of the family Caesalpiniaceae in West Africa in the early 1900s (Delobel 1995). In the American tropics and Hawaii it has not shifted to peanuts (Robert 1985, Delobel 1995, Nilsson and Johnson 1992). The confirmed host species are *Bauhinia monandra*, *B. rufescens*, *B. variegata*, *Cassia sieberiana*, *Piliostigma thonningii*, *P. reticulatum*, *Tamarindus indica*, and *Arachis hypogaea* (Pierre and Huignard 1990, Nilsson and Johnson 1992, Delobel 1995). An additional seven host species and another host family are recognised in earlier studies; *Acacia tortilis* and *A. modesta* (Wali-ur-Rehman 1993), *A. nilotica* (Singal and Toky 1990, El Atta 1993) *A. gerrardii* (Mucunguzi 1995) (Mimosaceae spp.), *Cassia arereh* (Robert 1985), *Prosopsis cinerea* and *P. juliflora* (Vir and Jindal 1996) (Caesalpiniaceae spp.).

While *Caryedon serratus* is considered to have originated in Africa, its distribution now extends to the tropics of Asia and the New World (Robert 1985) as well as Hawaii (Nilsson and Johnson 1992). The wide dispersal is thought to have been aided by anthropogenic distribution of its primary host plant, the tamarind (*Tamarindus indica* Caesalpiniaceae) the pulpy pod wall of which is used by people for food and juice (Robert 1985). In the New World tropics, the introduction of the bruchid may also have been aided by introductions of another host species, the ornamental tree *Bauhinia variegata*, from Asia (Nilsson and Johnson 1992).

The life cycle of *Caryedon serratus* has been described for various host species including peanut (Feakin 1973, Kokalis-Burelle *et al.* 1997), *Bauhinia rufescens* (Pierre and Huignard 1990), *Acacia nilotica* (El Atta 1993) and *Piliostigma thonningii* (Gagnepain and Rasplus 1989). In *A. nilotica,* for example eggs are laid on the surface of the pod and after 7-16 days the larvae burrow into the seed to feed before emerging to pupate in a cocoon (El Atta 1993). Infestation is greatest in stored seed, intermediate in pods on the forest floor and least in pods on the tree. The egg to adult development time is around 42 days after which the adult lives for 4-15 days, with mating beginning after one day and egg laying within two to three days. Pfaffenberger (1985) provides a checklist of citations with illustrations and/or descriptions of first and final larval stages of *C. serratus*. Illustrations of the abdomens of male and female adults are presented in Davey (1958) and photographs of cocoons, larvae and an adult (Feakin 1973) and larvae (Kokalis-Burelle *et al.* 1997) have been published.

Scanning electron micrographs of the eggs are presented in Wightman and Southgate (1982) as an identification aid for the bruchid.

Apart from a brief mention in Lawrence and Britton (1994 p. 152), the occurrence of *C. serratus* has not been formally noted in the Australian fauna. It is not known how the bruchid was introduced into Australia. The Australian National Insect Collection (ANIC) includes Australian collections from as early as 1947 (Table 5.1). The collection sites point to anthropogenic spread of the bruchid into and within Australia with seeds of ornamental or fodder trees.

Table 5.1. Australian National Insect Collection (ANIC) specimens of *Caryedon serratus* up to 1999.

Locality	Date	Collector	Host plant
1. Townsville	01 Feb 1947	SRE Brock	
	10 Jun 1948		
	23-31 Jul 1967	P Ferrar	
2. Tindal	01-20 Jul 1967	WJM Vestjens,	
3. Edge Hill, Cairns	Jun 1966	JG Brooks	
	01 Oct 1966		
	22 Feb 1967		'cassia seeds'
	14 May 1967		bred from 'yellow cassia'
4. Bowen	15 Jan 1967	JG Brooks	tamarind
	Jan 1979		*Acacia nilotica* seed pods
5. Mareeba	12 Jul 1986	H & A Howden	
6. Ex lab colony, CSIRO Long Pocket	28 May 1969	B Champ	
7. Canberra	24 Jun 1971	T Bellas	
8. Farrar, Canberra	21 Jan 1992	DCF Rentz	*Albizia* sp.

Predation of seed of native *Cassia* species by *Caryedon serratus* has not been previously reported in Australia. Jones and Elliot (1986) report several insects associated with Australian *Cassia* species in general, and the occurrence of larvae of the 'Cassia caterpillar' (*Catopsilia* spp.) feeding on leaflets of *Cassia brewsteri*, *C. marksiana* and *C. queenslandica*. Williams (1980) noted frequent damage to the seeds of *C. brewsteri* by insect larvae and beetles, although the insects were not specified.

Davey (1958) listed 42 plant species reported to be attacked by *Caryedon serratus* (*C. gonagra*) including a record of the bruchid on *Cassia brewsterii* in 1952 in the Smithsonian collection, however this record is flawed. The nomenclature "*Cassia brewsterii*" has never been formally adopted in the botanical literature. Mueller used "Cassia Brewsterii", in an 1858 checklist of plants before publishing the first formal description of *Cassia brewsteri* (as *Cathartocarpus Brewsterii*) in 1859. In Bentham's (1864) *Flora Australiensis*, the name *Cassia brewsteri* was first used and has been since. Further, the location of the collection site for the Smithsonian's *Caryedon serratus* / "*Cassia brewsterii*" record was not given. As noted above, *C. brewsteri* is endemic to central Queensland, however Davey (1958) noted that the insect was absent from Australasia. Given the uncertainty of the circumstances of this collection it cannot be concluded that it represents a record of the bruchid's occurrence on *C. brewsteri in vivo*.

The aims of the present study were firstly to formally note the presence of *Caryedon serratus* in Australia and its potential threat to native *Cassia*, and secondly to assess the risk of spread to commercial peanut crops.

Materials and methods

Population sampling

Pods of *Cassia brewsteri* were collected from trees on 36 sites throughout the natural distribution of the plant in January 1998, approximately two to four weeks after ripening. Further collections were made from nine sites in January 1999, including two cultivated trees outside of the natural distribution. Collections of *C. tomentella* pods were made in 1998 (three sites) and 1999 (one site), where the distribution of this species approached that of *C. brewsteri*. For each site, one to five fruit-bearing trees

were selected and samples of up to 20 pods collected from each tree. The sample size depended on the number of fruit-bearing trees on site and the fruit yield per tree. In practice, each site yielded between one and 54 pods (median 13).

Level of infestation of seed

The bruchid was identified using representative samples of both adult and larval stages. Specimens of *Caryedon serratus* previously deposited in the ANIC were also verified (Chris Reid).

The proportion of trees affected in each locality was assessed by examining the stored pods from each tree for larval exit holes and cocoons (approximately one month after the collection date). The proportion of pods affected was assessed for one tree per site. The proportion of seeds destroyed was assessed for one individual pod from this tree (21-53 seeds, median 29), by removing and examining all seeds. The infestation results were reported as the mean ± the standard error.

Life cycle

Larvae were observed with the aid of a dissecting microscope. Adults (20) were kept in a plastic bin and presented with intact pods of *Cassia brewsteri*, tamarind, and peanuts (whole and shelled). To prolong the life of the adult bruchids, a saturated aqueous mannose solution was provided (Mital 1971). A second bin was set up with 10 adult bruchids and 10 ripe seeds of *C. brewsteri*.

Predicted distribution

The 21 new records and five historical records were geocoded and a bioclimatic model was created using the methods described in Chapter 3. Of the historical distributions, only localities one to five (Table 1) were considered to be naturalised occurrences. The collections from sites in Canberra and Brisbane were interpreted as isolated occurrences, probably representing introductions in ornamental tree seed, and not established breeding populations.

Results

Distribution of Caryedon serratus on Cassia brewsteri

In 1998, 13 of 36 *Cassia brewsteri* sites and one of three *C. tomentella* sites were infested with *Caryedon serratus* (Fig. 5.1). In 1999, six of nine *Cassia brewsteri* sites and the one *C. tomentella* site were infested. Overall, *C. serratus* was collected at 21 sites, and not detected at 25 sites.

The distribution of the insect was limited to the northern part of the range of *C. brewsteri* (latitudes 19.258 - 24.140° S). Occurrences on *C. tomentella* extended this range as far south as 24.427° S. The entire range of *C. tomentella*, which extends as far south as 28.267° S, was not surveyed.

Level of infestation of seed

The collection sites in the southern part of the distribution of *Cassia brewsteri* (c. 26° S) were typically low in pod yield, three sites with many mature trees yielded only one to seven pods in total in 1998 and no pods in 1999. The northern-most range of the plant bore high pod yields in both 1998 and 1999.

On sites where *Caryedon serratus* was recorded, the proportions of trees infested averaged 71 ± 8.5% and ranged from 25 to 100%. The proportion of pods affected per tree averaged 72 ± 8.6% and ranged from 12.5% to 100%. The proportion of seeds destroyed per pod averaged 40 ± 8.0% and ranged from 1.9% to 83.3%. Small and variable sample sizes did not allow for a statistically valid comparison of seed predation rates between sites. A small sample was adequate for detection of the insect, e.g. a single fruiting tree and a collection of six pods for a given site.

Life cycle

Larvae began to emerge through 2-3 mm holes in the testa and pod wall of the mature, dry *Cassia brewsteri* pods, one to four weeks after pod collection. The entire endosperm and embryo of infested seeds was eaten, leaving only the testa. Oval papery cocoons were formed on the surface of the pods or the inside of the plastic storage bags. Within several days of emergence from the cocoon, adult beetles laid translucent oval eggs (c. 1-2 mm in diameter) on the outside of pods. Larvae from the eggs were observed to bore directly into the pod and through the disc of dry suberous tissue surrounding the seeds.

Adult bruchids were presented with a range of seeds and pods as potential substrates on which to develop. Although daily observations were not made, the time from oviposition to adult emergence was approximately the same as that previously reported for the species, i.e. around seven weeks. A complete life cycle (adult to adult) was observed in six of ten *C. brewsteri* seeds presented to an adult bruchid. *Caryedon serratus* raised on *Cassia brewsteri* pods also developed successfully on tamarind and peanuts (shelled and whole) *in vitro*. When several substrates were presented simultaneously there was a distinct preference for oviposition on *C. brewsteri* pods (8.5 eggs/cm on 38.6 cm^2) compared to tamarind pods (3.9 eggs/cm on 30.5 cm^2), peanut pods (1.3 eggs/cm on 22 cm^2) and shelled peanuts (2.3 eggs/cm on 12 cm^2).

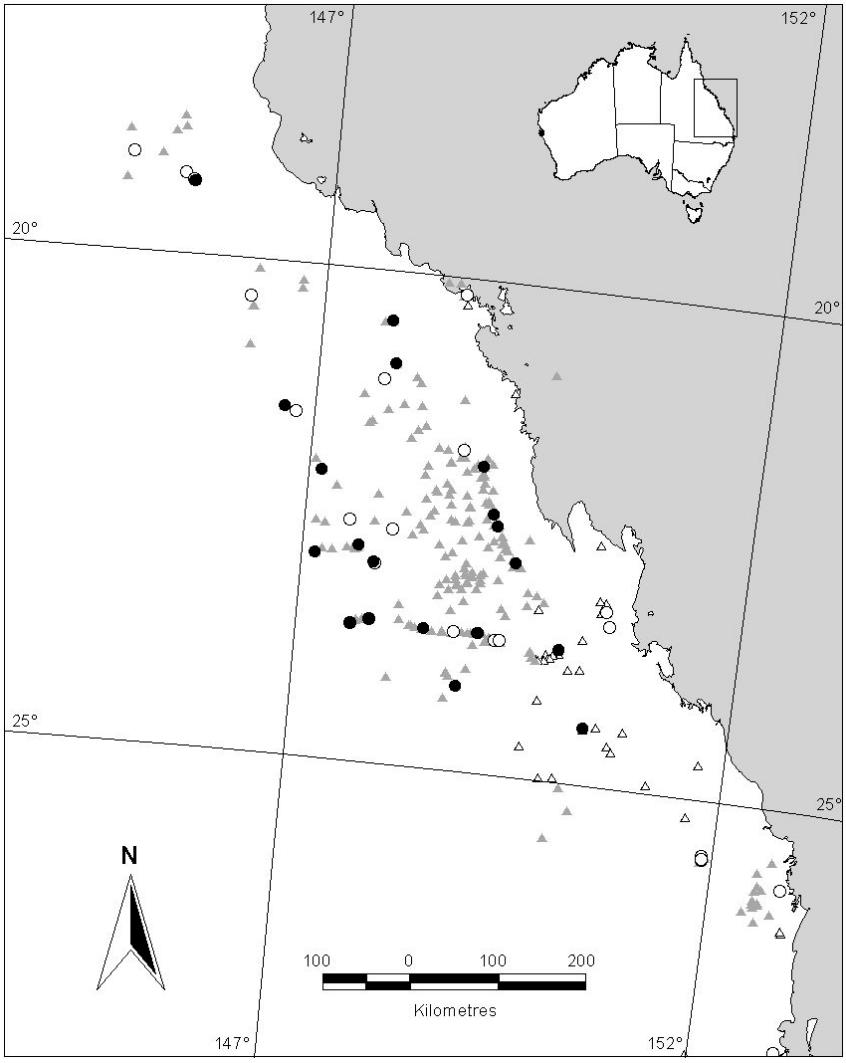

Figure 5.1. Distribution of *Caryedon serratus* on two host species *Cassia brewsteri* and *C. tomentella* (1998 and 1999). *C. serratus* present (solid circles), *C. serratus* absent (hollow circles), *C. brewsteri* distributions (grey triangles), *C. tomentella* distributions (hollow triangles).

Predicted distribution

New distributions on *Cassia* (21) and historical distributions from the ANIC (five) were used to generate a bioclimatic profile for 35 bioclimatic parameters (Table 5.2). The predicted distribution of the plant based on three selected bioclimatic parameters is a broad swathe across the dry tropics of Australia and a small area in coastal, southern Western Australia (Fig. 5.2).

Table 5.2. Bioclimatic profile for 26 recorded distributions of *Caryedon serratus* in northern Australia.

Parameter		Mean	S.D.	5%	50%	95%	Max.	Min.
1	Annual mean temperature	22	1.27	20.6	21.8	26.1	26.1	20
2	Mean diurnal range (mean(monthly max-min))	13.1	2.11	8.6	14	15.1	15.1	8.1
3	Isothermality (2/7)	0.53	0.02	0.51	0.53	0.58	0.58	0.5
4	Temperature Seasonality (C of V)	1.44	0.26	0.94	1.56	1.73	1.73	0.82
5	Max temperature of warmest month	33	1.65	30.9	32.9	36.9	36.9	30.8
6	Min temperature of coldest month	8.4	2.98	5.5	7.5	16.7	16.7	5.3
7	Temperature annual range (5-6)	24.6	4.03	16.4	26.2	28.9	28.9	14.5
8	Mean temperature of wettest quarter	26.5	0.84	25.2	26.8	28.5	28.6	24.9
9	Mean temperature of driest quarter	17.7	1.93	15.7	16.9	21.7	21.7	14.9
10	Mean temperature of warmest quarter	26.6	0.92	25.3	26.7	29.5	29.6	25.2
11	Mean temperature of coldest quarter	16.3	2.04	14.2	15.7	21.4	21.4	13.7
12	Annual precipitation	757	321	539	673	2126	2184	524
13	Precipitation of wettest month	151	82.5	102	127	461	461	99
14	Precipitation of driest month	15	7.3	2	16	35	36	0
15	Precipitation seasonality (C of V)	74	19.6	54	68	115	116	52
16	Precipitation of wettest quarter	408	230	264	347	1342	1342	255
17	Precipitation of driest quarter	57	22.9	8	61	109	112	0
18	Precipitation of warmest quarter	376	173	262	324	1065	1065	255
19	Precipitation of coldest quarter	71	23.3	33	74	134	138	4
20	Annual mean radiation	20	0.52	19.3	20	21.7	21.8	18.7
21	Highest monthly radiation	25.1	0.36	24.5	25.2	25.6	25.6	23.9
22	Lowest monthly radiation	14.2	0.93	13.3	14	18.4	18.4	13.2
23	Radiation seasonality (C of V)	19	1.9	17	19	20	20	10
24	Radiation of wettest quarter	22.4	1.24	19	22.8	23.4	23.4	18.6
25	Radiation of driest quarter	18.2	0.97	17.4	18	21.2	21.2	17.3
26	Radiation of warmest quarter	22.8	0.7	21	22.9	24.3	24.3	20.5
27	Radiation of coldest quarter	15.7	0.9	14.9	15.6	19.7	19.7	14.8
28	Annual mean moisture index	0.39	0.14	0.27	0.37	0.91	0.91	0.26
29	Highest monthly moisture index	0.56	0.18	0.4	0.5	0.98	1	0.39
30	Lowest monthly moisture index	0.22	0.11	0.12	0.2	0.67	0.69	0.08
31	Moisture index seasonality (C of V)	29	10.6	16	27	60	62	12
32	Mean moisture index of high quarter MI	0.53	0.18	0.37	0.48	1	1	0.36
33	Mean moisture index of low quarter MI	0.24	0.11	0.13	0.23	0.73	0.75	0.12
34	Mean moisture index of warm quarter MI	0.4	0.12	0.25	0.38	0.81	0.83	0.21
35	Mean moisture index of cold quarter MI	0.41	0.16	0.28	0.35	0.96	0.98	0.27

Discussion

Life cycle

The duration of the combined four larval stages of *Caryedon serratus* is reported as 41.7 days in *Acacia nilotica* at 35 ± 5°C (El Atta 1993) and 42 days in peanut at 30 °C (Feakin 1973) (relative humidity was 70% in both cases). Adult longevity of *C. serratus* in *Acacia nilotica* was 4 to 15 days (El Atta 1993). These life cycle periods indicate that there are potentially six generations of bruchids each year.

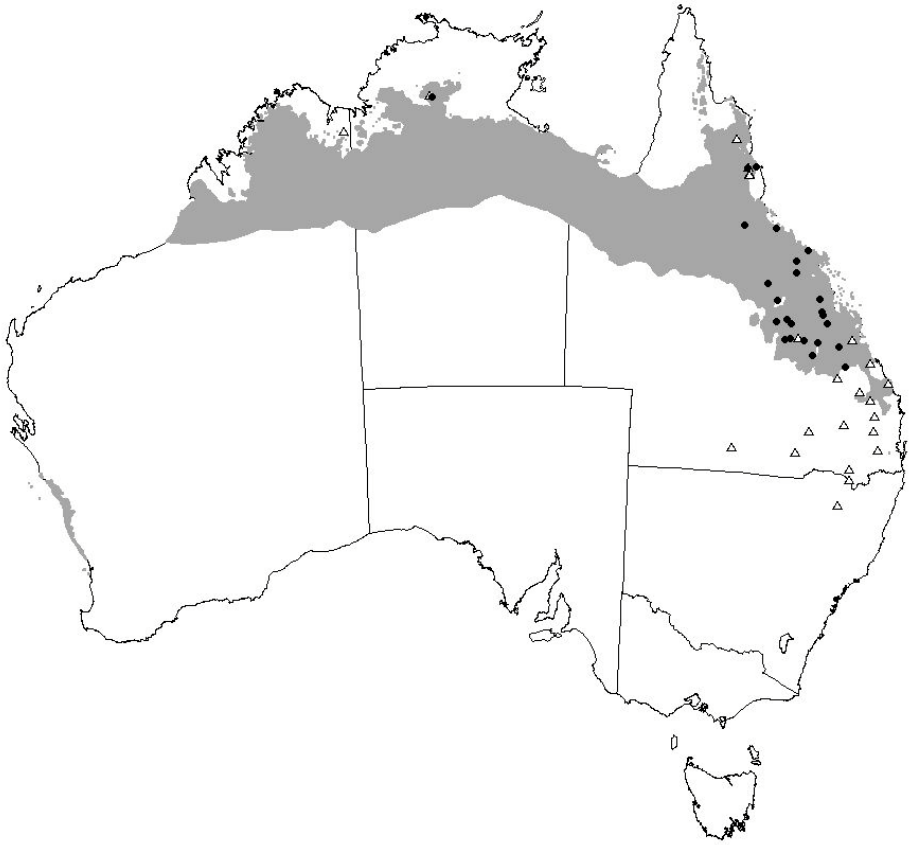

Figure 5.2. Predicted distribution of *Caryedon serratus* in Australia based on 26 distributions and three bioclimatic parameters: precipitation of Driest Quarter (0 - 112 mm), precipitation of Wettest Quarter (255 - 1342 mm) and mean temperature of coldest quarter (13.7 - 21.4 °C). Solid circles show historical and new distributions, hollow triangles show localities of major peanut cropping areas in Australia.

The timing of larval emergence from pods collected in January suggests that eggs were laid on *Cassia brewsteri* and *C. tomentella* in mid December, a time when most pods are still green. However, secondary infestation was observed in stored pods of *C. brewsteri*, which suggests that successful development can occur in this host species when eggs are laid on dry, ripe pods. *Caryedon serratus* is known to lay eggs only on brown dehydrated pods of *Bauhinia rufescens* (Pierre and Huignard 1990). In *Acacia nilotica* and *Piliostigma thonningii*, infestation begins on developing pods and subsequent generations develop on ripe pods that remain on the tree (Vir and Jindal 1996).

Mature *Cassia brewsteri* pods can, rarely, be found on the parent tree or nearby ground throughout the year and may thus provide a substrate for the bruchid for the entire year. However, the size of the bruchid population surviving on this resource would be small, and a rapid population buildup of *Caryedon serratus* must then occur once substrate is readily available to account for the high observed levels of *Cassia brewsteri* seed predation.

Hosts and historical geographic range

From ANIC collections, *Caryedon serratus* has been present in Australia since at least 1947. Nilsson and Johnson (1992) speculate that anthropogenic spread of seed of *Bauhinia variegata*, a common ornamental tree, and tamarind, a common ornamental and food tree, may function as a means of dispersal for *C. serratus* in the American tropics. *C. serratus* may also have been introduced to Australia through imported seeds of tamarind and ornamental bauhinia species. Macassan fishermen introduced tamarinds into northern Australia (Ross 1998), thus *C. serratus* may have been established in Australia centuries before European settlement. If this were so, *C. serratus* should occur in north west Western Australia (W.A.) and the Northern Territory (N.T.). However, there are no records of collections in W.A. and only one collection from the N.T. presently held in the ANIC. The absence of

records from W.A. and other northern regions may reflect the *ad hoc* nature of collections of museum specimens and does not imply that the insect is absent from that state.

C. serratus is known to occur on *Acacia nilotica* (El Atta 1993). *A. nilotica*, or prickly acacia, is an introduced plant which has become a widespread weed in western Queensland, suppressing pasture growth and spreading rapidly by seed. None of the Australian collections refer to specimens collected from this tree. This may be due to the paucity of Australian collections. However, if *C. serratus* has not yet spread to *A. nilotica,* the insect may still be spreading west to areas in which *A. nilotica* is naturalised. In either case, a beneficial aspect of *C. serratus* establishment may be the biological control of *A. nilotica* in Australia.

The ANIC records include locations in the urban areas of Townsville and Cairns. It is possible that these collections are a result of multiple introductions via commercial plant nurseries. The two ANIC collections from the temperate Canberra region represent an unusual result, and probably represent anthropogenic spread via transport of infested seed. This could result in a short-term population relying on opportune climatic conditions, rather than an established breeding population.

Other potential host species are the exotic *Albizia lebbeck* (Caesalpiniaceae) and native *Albizia* species, which occur commonly in central Queensland. Similarly, *Cassia fistula* is widespread in cultivation and has become semi-naturalised in Queensland. *C. fistula* is sometimes traded in plant nurseries as 'yellow cassia' and may account for Brooks' 1967 collections of *Caryedon serratus* on 'yellow cassia' in Cairns.

Impact of parasitism on *Cassia brewsteri* seed

The level of seed predation by *Caryedon serratus* on *Cassia brewsteri* is significant at c. 40% and will presumably be affecting the recruitment of seeds to soil seed banks, and thus impacting seedling establishment. If this hypothesis is correct, a major long term impact on *C. brewsteri* ecology can be expected, in terms of seedling recruitment.

Validation of predicted distribution

The *Caryedon serratus* distribution model requires further validation. Bioclim-compatible climate surfaces are becoming available for countries other than Australia, including Africa for example, so a predicted distribution for Africa could be compared with actual collections of the insect. Another approach would be to selectively ground truth the model by conducting further collection trips, for example in western Queensland where other potential host species occur.

Potential to parasitise peanuts in Australia

In India, *Caryedon serratus* is reported to infest peanut, with *Acacia nilotica*, *Prosopis cinerea* and *P. juliflora* acting as seasonal hosts (Vir and Jindal 1996). In Australia, the northern peanut cropping areas overlap the actual and predicted potential distribution of the bruchid (Fig. 5.2). In Central Queensland, peanuts are planted from mid October until the end of January and harvested between February and July (Garner and McRuvie 1996). Thus, harvested peanut crops should be vulnerable to the emergence of adult *C. serratus* from *Cassia* pods occurring from January to February. There is no evidence, however, to suggest that *C. serratus* has shifted to peanuts in Australia. It is not mentioned as a pest of Australian peanuts in Garner and McRuvie (1996) or Crosthwaite (1994) and it is also absent from lists of insect pests of peanuts in the South Burnett (Anon. 1997a) and Darling Downs (Anon. 1996) regions.

Subspecific variation in morphological (Sembene and Delobel 1996) and genetic (Sembene and Delobel 1998) characters in *Caryedon serratus* has been linked to host specificity. Thus, the failure of *C. serratus* to infest peanuts in Australia may be due to the particular variety that has become established in Australia. However, the *in vitro* results reported above suggest that bruchids raised on native *Cassia* species can breed in peanuts despite the fact that this has not been reported in a cropping situation.

Robert (1985) found that females of *Caryedon serratus* from *Bauhinia rufescens* (in an area with no peanuts) and from peanuts (in an area with few wild hosts) preferentially laid eggs on pods of their original host plant when alternative substrates were also available. In the current study, *C. serratus* reared from *Cassia brewsteri* seed were observed to lay eggs on pods of *C. brewsteri,* tamarind and peanut pods, however there was a marked preference for oviposition on pods of the parental host species, in this case *C. brewsteri*. Chance events favouring oviposition on peanuts could build up a population of *Caryedon serratus* that favour peanut as a host. Given the speculative element of the above conclusions, it is recommended that further work on the potential for *C. serratus* to infest peanut in Australia be undertaken.

6. Galactomannan content and composition in *Cassia brewsteri* seed

Abstract

The galactomannan content of 40 samples of *Cassia brewsteri* seed from throughout the distribution of the species ranged from 28.3% to 39.7% of seed dry weight. The average galactomannan content was 33.7 ± 0.4%. The mannose to galactose ratio ranged from 4.6 to 6.3 and averaged 5.43 ± 0.06. *C. brewsteri* gums prepared in a manner analogous to carob gum were tested for gel strength, colour and clarity, relative to carob gum. The break force index (BFI) of *C. brewsteri* gum / carageenan relative to carob gum / carageenan ranged from 56% to 117%. The BFIs of two of the *C. brewsteri* gum preparations relative to *Senna tora/obtusifolia* gum were 83% and 93%. The variability in the results was attributed to differences in sample preparation and to variability in the galactomannan characteristics of the seedlots used for gum production. The colour and clarity of *C. brewsteri* gum preparations were considered adequate by industry standards.

Introduction

Non-starch carbohydrate reserves such as galactomannans, mannans, glucomannans and galactoxyloglucans are generally deposited in the cell walls of endosperm tissue or cotyledons. Many leguminous plants species with a copious thick-walled endosperm store galactomannans in the cell walls of the endosperm. Galactomannan-based endosperm can absorb several times its own dry weight in water, thus the endosperm is able to provide a water reservoir for the germinating embryo. After germination, the galactomannan is catabolised and the products transferred to the embryo supplying carbon and energy. For these reasons it has been described as a dual-purpose polysaccharide (Dea and Morrison 1975, Reid and Bewley 1979, Hegnauer and Grayer-Barkmeijer 1993).

Many seed galactomannans consist of polysaccharides containing a (1→4)-β-D-mannan backbone with single (1→6)-α-D-galactose side-chains linked to some of the D-mannosyl residues. (Dea and Morrison 1975). The galactomannans extracted from different species and within the one seed sample vary in the ratio of mannose to galactose. Hence a gum sample is composed of a number of different gums with different two and three-dimensional arrangements and properties (Dea and Morrison 1975, Fox 1997).

Several authors have pointed to differences in chemical composition of the galactomannan reserves in the three leguminous plant families (Reid and Meier 1969, Buckeridge and Dietrich 1990, Hegnauer and Barkmeijer 1993, Buckeridge *et al.* 1995). The ancestral group Caesalpiniaceae has consistently higher galactomannan content and a higher mannose to galactose ratio than the Mimosaceae and Fabaceae. These differences are thought to reflect adaptations through different strategies to tropical and arid environments.

The main sources of galactomannans for industry are carob (*Ceratonia siliqua*), native to Mediterranean countries, and guar (*Cyamopsis tetragonolobus*), a native of India and Pakistan. Many other species have been examined for commercial potential since the 1940s, with at least 120 species reported in the public literature (data not shown) and many more likely to have been analysed but not reported. However, very few species have achieved any commercial impact on the galactomannan market. One exception is gum sourced from *Senna tora* (*Cassia tora*) and *S. obtusifolia* (*C. obtusifolia*). Another new gum becoming traded on world markets is tara gum which has recently been produced in small amounts from the seeds of the tara tree, *Caesalpinia spinosa*, in Peru.

Seed galactomannans are used in a wide range of industrial applications to form a highly viscous aqueous solution by themselves or an aqueous gel in synergy with other polysaccharides. Some of the many foods containing galactomannans are ice cream, other milk-based products and desserts, mayonnaises, dressings, sauces and deep-frozen foods. Non-food uses of galactomannans include

lubrication of oil drills, waterproofing of underwater explosives and paper and textiles manufacturing. (Dea and Morrison 1975). Carob, guar and *Senna tora/obtusifolia* gums are used in many industrial applications because they are far less expensive than other thickeners like carrageenan and agar. Different combinations of seed gums and other polysaccharides are used to achieve different functional requirements in both processing and/or final presentation of many products.

The quality of gum used in industrial processes varies greatly from crude flour made by grinding whole seeds to purified endosperm extracts with less than 2% contaminants (testa and embryo). The disadvantages of the crude extracts are less stability and clarity in solutions and unacceptable flavour in foodstuffs (Dea and Morrison 1975). Typical gum yields for carob seed are 45-50% (w/w) (food grade), 55-65% (technical grade) and 70-90% (pet food grade) (Race *et al.* 1999).

The aims of the present study were to investigate variability of *Cassia brewsteri* seed galactomannan content and mannose to galactose ratio, and to determine whether the galactomannan had functional characteristics suitable for industrial applications as a gelling agent.

Materials and methods

Fruit samples were collected from throughout most of the range of *C. brewsteri* in December 1997, and January and February 1998. Herbarium specimen distributions (mainly BRI) and new collection sites were mapped in ArcView (ESRI 1996) and presented in Albers equal-area conic projection (Fig. 6.1). Seeds were extracted from one to seven pods from each individual tree, a total of 44 seedlots from 40 trees across the distribution of the species including 10 from trees on a single site ('Longdale') and four from a single tree.

Uniform seed (4 g) was separated from the pods by hand with atypical (discoloured, deformed, unusually small or large) seeds being discarded. Galactose and mannose content and composition were determined after the method of Hansen *et al.* (1992) with the following modifications: seed was ground to < 1.5 mm in hammer mill, dried at 80 °C for 24 hr and ground in a ZM1000 grinder to < 0.12 mm; seed powder (0.2 g) was hydrolysed with 1M trifluoroacetic acid in 3 mL micro reaction vessels with solid caps with polytetrafluoroethylene liners (Supelco 2-7038); and mannose and galactose content of the aqueous hydrolysate solutions was determined using a Supelcosil LC-NH2, 5 µm, 250 × 4.6 mm (ID) aminopropyl bonded phase column (Supelco 5-8338) with a Waters 717 autosampler and an ERMA RI detector (ERC 7510). The mobile phase was acetonitrile:water (80:20) at 1.0 mL/min. Sugars were identified by comparison with the retention times of authentic standards. Quantitation was by peak area integration using Delta 4.26B software (Digital). Quantitative results were reported as means and standard errors of the means.

C. brewsteri gum samples were prepared by manually extracting whole endosperms from hydrated seed then grinding them in a hammer mill to < 1000 µm. To enable hydration, the testa was first breached by either freeze-drying at -100 °C or treatment with concentrated H_2SO_4 for 20 min. Two gum samples were prepared from bulk seedlots from the Blackwater region of Central Queensand. The bulk of the endosperm tissue (c. 70%) was ground to < 425 µm, the remaining material was extremely hard and could not be ground to this mesh size without burning. This fraction, at > 425 µm and < 1000 µm, was tested separately.

Blocks of gel were prepared as an aqueous mixture of the seed gum sample and carrageenan (each at 0.5% w/v). Gel strength was measured with a FIRA jelly tester and expressed as a break force index (BFI). The BFI is the weight of water required to rotate the jelly tester's blade inserted in the gel block through 90° expressed as a percentage of the weight required for a block of standard gel prepared at the same time. *C. brewsteri* gums were assessed for BFI relative to a carob gum standard and (where enough sample was available) a *Senna tora/obtusifolia* standard. Colour and clarity were assessed visually using the gel blocks prepared for BFI testing.

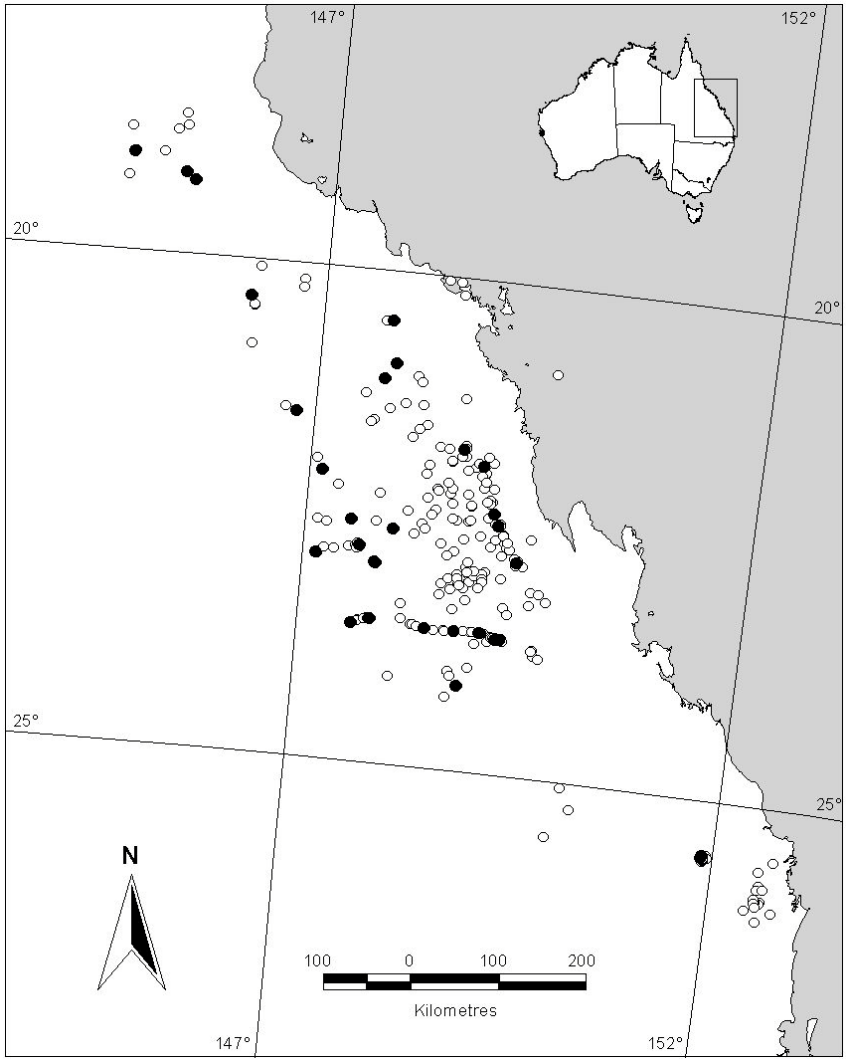

Figure 6.1. Natural distribution of *Cassia brewsteri* (hollow circles) and collection locations of the 40 seedlots analysed for galactomannan content and composition (solid circles).

Results

The galactomannan content of 40 samples of *C. brewsteri* seed collected across the geographic range of the species ranged from 28.3% to 39.7% (w/w) (representative seeds, Fig. 6.2). The average galactomannan content was 33.7 ± 0.4% (Table 1). The mannose to galactose ratio ranged from 4.6 to 6.3 and averaged 5.4 ± 0.1. The 'average' structure of the galactomannan polymer therefore consists of a mannose backbone with a galactose residue approximately every five mannose units. There were no clear trends in the geographical distribution of galactomannan content or Man:Gal (visually assessed in ArcView, data not shown).

The galactomannan content of samples from ten trees on one site ranged from 31.6% to 39.7% and averaged 35.4 ± 0.9%. In samples from four pods on a single tree the galactomannan content ranged from 30.6% to 36.3% and averaged 32.7 ± 1.2%. The mannose galactose ratio within the one site ranged from 5.1 to 6.3 and averaged 5.5 ± 0.1. Within one tree, the mannose to galactose ratio ranged from 3.6 to 5.4 and averaged 4.7 ± 0.4. The high level of variability between pods on the one tree ws due to one outlying data point (in both galactomannan content and mannose to galactose ratio). This outlier may be due to that pod being at a different stage of ripening.

A sample of seeds (20 g) yielded 55.0% (w/w) endosperm, 28.5% testa and 16.5% embryo. *C. brewsteri* gum samples were a pale cream colour without contamination by flecks of seed coat or embryo (Fig. 6.3). The BFI of one whole ground endosperm sample was 107 (relative to carob) and 83

relative to *S. tora/obtusifolia* gum) (sample F, Table 6.1). The colour and clarity of *C. brewsteri* gum preparations were comparable to commercially produced carob and guar gums.

Table 6.1. *Cassia brewsteri* gum quality assessment expressed as galactomannan content (M+G), mannose to galactose ratio (M:G) and Break Force Index (BFI).

Sample	Description	M+G (%)	M:G	BFI (carob)	BFI (*S. tora*)
A	*C. brewsteri* whole seed (mean, 40 samples from across range)	33.7 ± 0.4	5.4 ± 0.1		
B	*C. brewsteri* whole seed (mean, 10 samples from one site)	35.4 ± 0.9	5.1 ± 0.1		
C	*C. brewsteri* whole seed (mean, 3 samples from one tree)	31.6 ± 0.6	5.1 ± 0.2		
D/1	*C. brewsteri* tree 66D endosperm < 425 µm (67% of total)	59.0	5.7	93	
D/2	*C. brewsteri* tree 66D endosperm > 425 µm (33% of total)	63.9	5.6		
E/1	*C. brewsteri* Blackwater bulk 96/97endosperm < 425 µm (69% of total)	67.0	5.2	96	93
E/2	*C. brewsteri* Blackwater bulk 96/97endosperm > 425 µm, < 1000 µm (20% of total)	67.8	5.0	117	
F	*C. brewsteri* 97/98 bulk collection endosperm < 1000 µm	64.3	3.9	107	83
S. tora	*Senna tora* standard (1998 Standard CS-98 2593025)	49.87	6.02	109	
Carob	World Carob Standard (1992 M 421 H/N 8636)	38.78	3.75		
Guar	Guar Standard (number 355 - undated)	72.16	1.69		

Discussion

Gum yield and quality

The average galactomannan content (33.7% w/w) and mannose to galactose ratio (5.4) of *C. brewsteri* seed is high relative to existing commercial seed gum sources making it a very promising candidate for exploitation as a source of industrial gums on a biochemical basis. The highest galactomannan content of 39.7% (w/w) for one *C. brewsteri* seed sample is higher than that previously reported for any other species. The galactomannan content of seeds of commercial gum crops is variable between provenances and between years. In both carob and guar, galactomannan yields from 18 to 38% have been reported (Bailey 1971, Dea and Morrison 1975, McCleary *et al.* 1985, Strickland and Ford 1994).

The high galactomannan content found in *C. brewsteri* seed is due in part to the high endosperm content, the one sample tested yielded 55% endosperm on a dry weight basis. In comparison, guar endosperm comprises from 32 to 50% of the seed weight (Wielinger 1990, Menon *et al.* 1972), carob endosperm 40 to 50% (Wielinger 1990) and tara endosperm 22 to 27% (Wielinger 1990, Anderson 1949). Analysis of a larger set of samples is required to determine the mean level and variability of endosperm content in *C. brewsteri*, and its response to different environmental conditions.

The high mannose to galactose ratio of 6.3 for one *C. brewsteri* seed sample is also higher than any previously reported, the next highest being 5.2 in *Sophora japonica* (Fabaceae) (Dea and Morrison 1975). The average mannose to galactose ratio of 5.2 in *C. brewsteri* seed puts in it the same range as *Senna tora/obtusifolia* gum at 5 to 6 (BFGoodrich n.d.) and higher than carob gum at 4.0 to 4.5 (Dea and Morrison 1975).

Variation in gum yield and quality

Seed galactomannan content and composition varies considerably within a species and this variability is also evident in manufactured gums (McCleary *et al.* 1985). The interaction of environmental and genetic factors and their influence on gum characteristics in commercial gum sources is poorly understood. Gum yield in guar is influenced more by environmental factors than genetic factors, with differences between years greater than the differences between locations (Das *et al.* 1983, cited in Murphy *et al.* 1996). Guar gum yield has been found to be highest when seed yield is highest and this is associated with the optimal sowing time (Jain *et al.* 1987). Seed yield can be effected by row spacing depending on the branching habit of the cultivar, with branching types capable of adjusting to

wider row spacings (Malik *et al.* 1981). However, row spacing does not directly effect gum yield as a proportion of the seed (Singh *et al.* 1978, Malik *et al.* 1981).

Galactomannan fine structure

Cassia brewsteri seed contains polysaccharides which contain high levels of mannose and galactose, which are localised in the endosperm and which interact with carageenan to form aqueous gels in the same way as carob and *Senna tora* seed galactomannans. The galactomannan polymer structure reported here could be verified by direct evidence such as methylation, partial hydrolysis or NMR analyses. Structural investigations of seed galactomannans from *Cassia fistula* and *C. javanica* have shown that they contain the same basic structure as commercial seed galactomannans, a mannan backbone with single galactose side-chains (Dea and Morrison 1975). Since these species are the types for the Genus *Cassia* and the Series *Obolospermae*, of which taxa *C. brewsteri* is a member, it is inferred that the same galactomannan structure is conserved in this species.

Mannose to galactose ratio is thought to be controlled either during biosynthesis of the polymer or by post-synthesis removal of galactose by α-galactosidase. Edwards *et al.* (1992), conclude that post-depositional modification may be characteristic of the Caesalpiniaceae, which generally feature high mannose to galactose ratios. In contrast, Fabaceae species with high galactose substitution have been found to have a constant mannose to galactose ratio throughout the development of the seeds, indicating biosynthetic regulation. Since *C. brewsteri* is a member of the Caesalpiniaceae and has a very high mannose content, post-synthesis removal of galactose is likely to occur in this species. A series of tests of mannose to galactose and α-galactosidase activity of seed at intervals from anthesis to ripening could confirm this hypothesis.

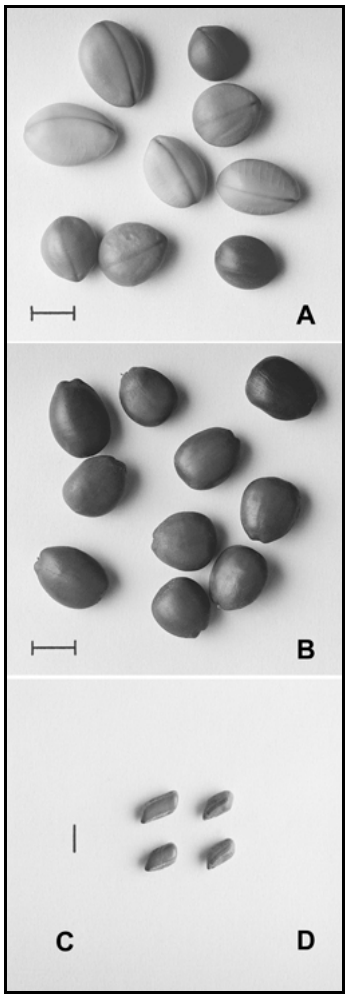

Figure 6.2. A) Cassia brewsteri, B) Ceratonia siliqua, C) Senna tora and D) Senna obtusifolia seeds.
(scale bars 5 mm except for *Senna* seed, 3 mm).

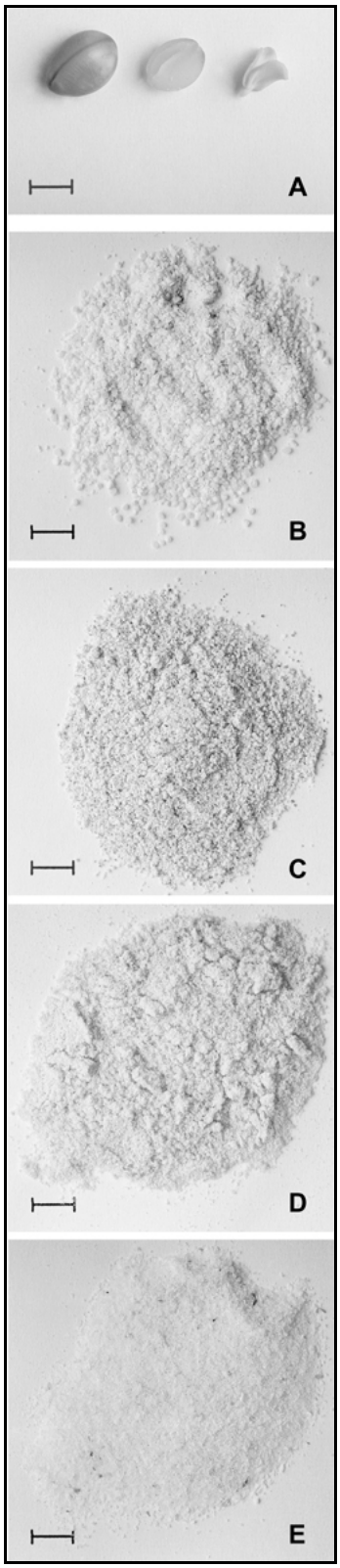

Figure 6.3. A) *Cassia brewsteri* seed, endosperm and embryo, B) *C. brewsteri* gum, C) carob gum, D) *Senna tora/obtusifolia* gum and E) guar gum.
(scale bars 5 mm).

7. Chrysophanic acid in *Cassia brewsteri* seed

Species of the genera *Cassia* and *Senna* containing anthraquinone glycosides are known for their medicinal uses, mainly as laxatives although few quantitative studies have been reported. *Cassia brewsteri* is unpalatable to cattle and hence is regarded as a weed by graziers. A report of the seeds of *Cassia brewsteri* having a purgative effect in humans (Leichhardt 1847) suggested that the seeds might contain anthraquinones. *C. brewsteri* fruit was assessed for chrysophanic acid to determine whether this compound was present in the seeds in excess of the 10 ppm set as the limit for *Senna tora/obtusifolia* gum (BFGoodrich n.d.). Alkaloid and anti-tumour testing of bark extracts (Collins *et al.* 1990) and digestibility and nutrient analyses of the leaves and twigs (Vercoe 1989) have been reported. *Senna obtusifolia* is known to contain anthraquinones (Van den Berg and Labadie 1989) and was used to corroborate the methods used.

C. brewsteri fruit was collected from west of Blackwater in January 1998. *S. obtusifolia* plants were collected from north of Mackay in August 1998. The plants were identified and voucher specimens (672252 and 672253) deposited in the Queensland Herbarium. *C. brewsteri* fruit was separated into pod wall, pith and seed. Ground and dried samples (5 g) were exhaustively extracted under reflux with 100 ml methanol. An aliquot was cleaned using a Sep-Pak C_{18} solid phase cartridge and free chrysophanic acid determined by HPLC after the method of (Van den Berg and Labadie 1985). A further aliquot (10 ml) of the seed extract was subject to oxidation and acid hydrolysis (Van den Berg and Labadie 1985) to assess total chrysophanic acid content (including glycosides, anthrones and dianthrones converted to aglycones).

Free chrysophanic acid was present in trace amounts in *C. brewsteri* seed (< 5 ppm) and pod wall material (5 ppm) but was undetectable in the pith (Fig. 7.1). Total chrysophanic acid in the seed was also < 5 ppm. Free chrysophanic acid in *S. obtusifolia* seed was 6 ppm and total chrysophanic acid 628 ppm, a result consistent with the 824 ppm reported for the closely related *S. tora* (Desai and Shukla 1978). The recovery rate of chrysophanic acid added prior to extraction was c. 100% for *C. brewsteri* seed and pod wall, and for *S. obtusifolia* seed, and 90% for *C. brewsteri* pith. Hence, glycosides or anthracene derivatives of chrysophanic acid are not likely to be responsible for the reported purgative effect of *C. brewsteri* seed. The level of chrysophanic acid in *C. brewsteri* seed is below the 10 ppm set by as the maximum allowable limit for *Senna tora/obtusifolia* gum.

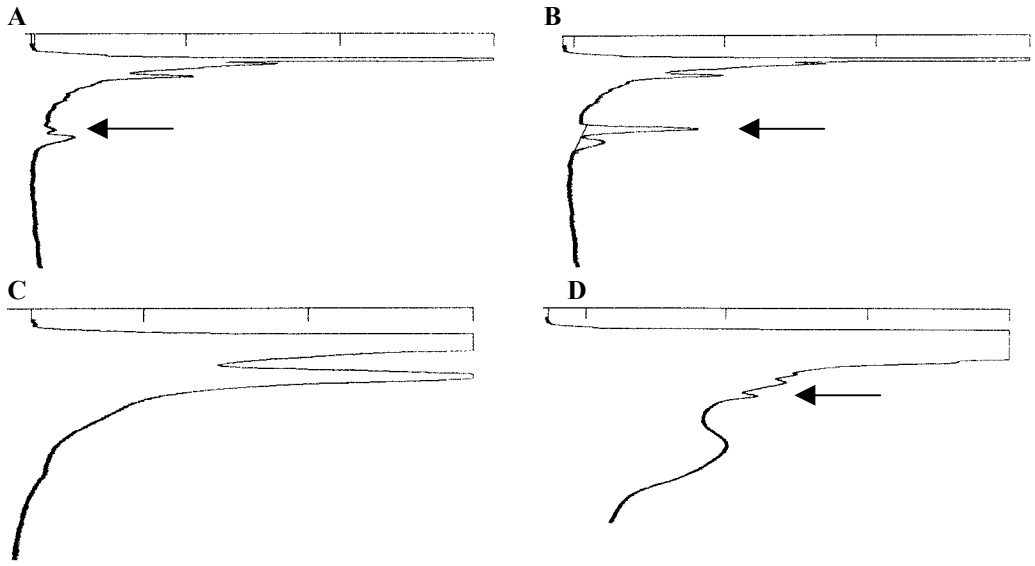

Figure 7.1. HPLC chromatograms of methanol extracts of *Cassia brewsteri* A) seed, B) seed + 20 ppm chrysophanic acid added, C) pith, and D) pod wall. The arrow marks the chrysophanic acid peak (retention time 4.1 min).

8. Commercial viability of *Cassia brewsteri* gum production

Abstract

Under the scenario of an existing farm enterprise converting some land to *Cassia brewsteri* orchards, the following returns per hectare are predicted for a 30 yr planning period using a financial model. Net Present Value (NPV) $331, Internal Rate of Return (IRR) 7.2%, benefit/cost ratio 1.020, payback period 29 yr and gross margin from year 16 onwards $2123. Sensitivity analysis of the model indicates that a 50% increase in the price of seed (from $1.60 to $2.40/kg) changes NPV to $8661, IRR to 11.5%, benefit/cost ratio to 1.530, payback period to 19 yr and gross margin from year 16 onwards to $3990. With processing costs estimated at $0.70/kg of gum, and a gum yield of 70% of seed mass, the price of *C. brewsteri* gum to the final user under this scenario would be $2.30 to $3.10/kg. This is competitive with indicative 1990s prices for pet food grade carob gum of $2.90 to $5.00/kg, however the returns to the grower under this scenario are not adequate to recommend cropping at this time. Cropping of the tree may be viable in future given adequate financial returns for environmental values such as salinity control and carbon credits.

Estimated returns and costs

Pod and seed yields and financial returns

Seed price: $1600/t, gum price: $2.30/kg

The value of a gum seed is determined by both the quality of extracted gum and the yield of useable gum per unit of seed. The yield of commercial gum per unit of seed is dependent on several factors: the galactomannan content of the endosperm; the endosperm content of the seed and the actual amount of endosperm and impurities extracted from the seed in the processing facility. The economic viability of seed gum production will depend on the price received per unit of seed, the seed harvest per unit of land area and the costs of production per unit area.

In an analysis of the commercial viability of carob gum production in Australia, Race *et al.* (1999) used a factory gate price of $1600/t of carob seed, based on the average cost of seed grown and delivered to processing mills in the Mediterranean in 1995 (cited in Curtis *et al.* 1998). Race (1997) previously estimated the price of carob seed at A$2000 to $2500/t which indicates the level of variability in carob gum prices. In 1996, the price of carob gum (technical grade) was A$2.00 to $2.50/kg (Race 1997) after reaching a high of A$32.00/kg due to crop and processing equipment failures in the major production areas of southern Europe in 1995 (Gebhardt 1996, CRCIBP 1996). The large fluctuations in the price of carob gum result directly from the uncertainty in supply of seed. This in turn is affected by two factors, annual variation in crop yields and the marketing strategies of the small number of companies controlling the seed market. A fundamental price for carob seed is thus difficult to determine.

For the current financial analysis of *C. brewsteri* seed gum, the factory gate estimate of $1600/t for carob seed was adopted, based on the earlier conclusion that the quality of *C. brewsteri* and carob gum was equivalent (Chapter 6). Gum processing costs for carob seed are estimated to be $500/t (Curtis *et al* 1998). The cost of processing per kg of gum (70% of seed, i.e. pet food grade gum) would therefore be 1/0.7 × $500 or $714/t of gum ($0.71/kg). With processing cost added to seed prices of $1.60 or $2.40, the price of *C. brewsteri* gum to the final user would be c. $2.30 to $3.10/kg. This price is competitive with indicative prices for pet food grade carob gum ($2.90 to $5.00/kg).

Carob (*Ceratonia siliqua*) has been grown in cultivation for around 4000 years. Like *Cassia brewsteri*, it is a relatively slow growing perennial tree with a woody pod. The carob fruit consists of only 10% (w/w) seed. While only the seed is used for gum production, the remaining 90% (w/w) is a pulp material high in sugar that can be used for stock feed or processed into powder or syrup for food additives (Esbenshade and Wilson 1986). It is unlikely that any such products could be made from

C. brewsteri pulp, as there are no reported cases of cattle eating the pods even in drought conditions (Chapter 7). *C. brewsteri* seed makes up around 35% (w/w) of the pod and is easily removed by mechanical means. This could be incorporated into the harvesting process to avoid unnecessary transport of low or zero-value material. The non-seed material could be left in the orchard as mulch to conserve nutrients and moisture on the site.

Seed yield: 2.3 t/ha

C. brewsteri seed yield per ripe pod (i.e. at field dryness) ranges from 10.8% to 44.7% (w/w) (mean 29.3%, n = 146 pods) (Chapter 3). Given that these data represent pods collected from wild trees, a crop seed yield of 35% is used in the financial analysis on the basis that seed filling under irrigation will produce yields which are higher in the observed range.

Experimental harvests of *C. brewsteri* pods from wild trees throughout central Queensland yielded 0.05 kg to 5 kg of pods from trees of manageable size (spreading habit, < 6 m high). These collections were undertaken in a low rainfall year of a low rainfall region. As any plantation would be grown under favourable conditions and with growing stock selected for high fruit yield, higher yields could be expected in cultivation. This financial analysis assumes a first viable harvest of 6 kg of pods/tree (2.5 t/ha) at around year 11, with yield increasing in 2 kg increments to 16 kg (6.5 t/ha) at year 16. Carob orchards in the Catalina region of Spain receive 300 L/yr of irrigation in addition to the 500 mm of rainfall and yield 6-7 t of pods per ha (or 24 kg pods/tree) after year 11 (Curtis *et al.* 1998).

The planting density used for carob in the Mediterranean, and proposed for carob in the Murray valley, is typically 6 m × 8 m to 6 m × 11 m, allowing for mechanical harvesting (Curtis *et al.* 1998). Olives in Australia are commonly planted at 5 m × 8 m (250 trees/ha) or 4.5 m × 7.5 m (296 trees/ha), with a minimum of 6 m between rows essential to allow access for mechanical harvesting (Holt and Bicknell 1998). *C. brewsteri* in Queensland could initially be planted at 6 m × 4 m (417 trees/ha). If fewer but larger trees proved to be a better management strategy the orchard could be thinned to 6 m × 8 m.

Financial returns: $3733/ha

Hypothetical scenarios for growing the tree in plantation culture and their resultant seed yield/ha are presented for a range of pod yields (6 to 16 kg/tree) at a planting density of 417 trees/ha. At a seed price of $1.60/kg, gross returns are estimated as increasing from $1400/ha to $3733/ha from years 11 to 16 under the assumptions developed above (Table 8.1). No consideration is given to products other than seed gums as it is assumed that the seed will be extracted on farm with the remaining pod components left on the site as mulch. Germ would be extracted at the processing mill and any commercial value of this product is included in the value assigned to seed.

Table 8.1. Hypothetical yield of a *Cassia brewsteri* orchard. Model assumes a row spacing of 6 m and spacing within rows of 4 m (i.e. 417 trees/ha), a seed yield/pod of 35%, a gum yield/seed of 70% and a price of $1.60/kg seed.

Pod yield (kg/tree)	Seed yield (kg/ha)	Return ($/ha)	Area (ha/t seed)	Gum yield (kg/ha)	Age (yr)
6	875	1400	1.14	613	11
8	1167	1867	0.86	817	12
10	1458	2333	0.69	1021	13
12	1750	2800	0.57	1225	14
14	2042	3267	0.49	1429	15
16	2333	3733	0.43	1633	16

A gum processing facility with a capacity of 1500 t seed/yr would fully utilise a modest 645 ha of *C. brewsteri* orchards growing under the above conditions. This would supply 1050 t/yr of pet food grade gum, Curtis *et al.* (1998) estimate the Australian demand for pet food grades of carob gum to be 1200 t/yr.

Light grazing could be carried out in the orchard to remove most of the expense of weeding and perhaps have an additional fertilising effect. Under the proposed tree spacing of 6 m between rows and 4 m spacing along the rows, an orchard with a square layout would have spaces equivalent to approximately 0.3 ha/ha grazing land. An estimate of the grazing value for a given site could be made by multiplying the full grazing returns for a similar land type by 0.31 (minus a factor to allow for shading effects). This return could be included from year 3 onwards to allow for adequate seedling development. However, in the current analysis, no value is assigned to the light grazing which could be carried out in the orchard.

Race *et al.* (1999) estimate that the return on carob timber would be $25/m and that if an orchard were scrapped it would yield 15 m^3/ha at year 20, 22 m^3/ha at year 25 and 30 m^3/ha at year 30. This estimate may be too high for *C. brewsteri* (or even carob), since the only useable sawlog would be the 0.8 to 1.2 m section of trunk pruned at the base to allow for mechanical harvesting. This timber could only be used for specialty furniture or carving applications. No value is assigned to the timber that could be recovered from a *C. brewsteri* orchard if it were to be scrapped at any time.

Estimated costs of orchard establishment and maintenance

Site preparation: $375/ha

The following financial analysis is based on an existing farming enterprise, with existing plant, switching to *C. brewsteri* cultivation. Hence, the only land cost is the opportunity cost of not using the land for other purposes. Survey and design was estimated at $50/ha, fencing at $205/ha and land preparation at $120/ha for carob (Curtis *et al.* 1998). These costs are used here as a first estimate but will vary with the type of land and the amount of preparation required.

Growing stock (seedlings): $417/ha

The price of tree seedlings and cuttings in NSW was reviewed by Bhati (1998) for orders of 10,000 to 20,000 plants in 10 cm long tubes. Seedlings raised from seed orchard trees and cuttings from selected pedigreed trees cost $0.29 (*Eucalyptus globulus*), $0.31 (*E. nitens*) and $0.17 (*Pinus radiata*). *P. radiata* cuttings cost $0.40. For this study, a costing of $1.00 per seedling is used as any production of *C. brewsteri* seedlings will be in lower numbers than these well established crop species. At 417 seedlings/ha, the initial growing stock would cost $417/ha. Any extra plants required to replace any lost plants after year one would incur additional costs. Cuttings of *C. brewsteri* have proved difficult to produce, and the only established methods of vegetative propagation are aerial layers or grafting onto young seedlings (Chapter 4). These methods may be too costly and time consuming for large-scale production. For example, the cost of grafted carob trees was estimated at $8/tree (Race *et al.* 1999), and commercial olive tree rooted cuttings cost around $4.50/tree (Holt and Bicknell 1998).

Irrigation system: $5000/ha

Installation of an irrigation system was estimated at $8000/ha for carob at 208 trees/ha (Curtis *et al.* 1998), although no breakdown of costs was given. The costing for an irrigation system for clumping bamboo (625 clumps/ha), adapted to allow for one sprinkler per *C. brewsteri* tree with 417 trees/ha, was $4432/ha (David Midmore, CQU, pers. comm.). Establishing an irrigation system for pawpaws was costed at $900/ha for 2000 trees (Anon. 1997b), although the type of plumbing used and the cost of labour was not specified. Given the wide range of price estimates for installation of an irrigation system in Central Queensland, a starting point of $5000/ha is used in the current analysis.

Irrigation water and system maintenance: $185/ha

The amount of water required for *C. brewsteri* can be based on a comparison with other tree crops. Irrigation levels of 0.009 and 0.04 ML/tree/year (averaging 25 and 110 L/tree/day) are recommended for pawpaw and lychee, respectively (Anon. 1997b, Hinton 1995). These water requirements are for plants yielding high moisture, pulpy fruit at up to 80 kg/tree (e.g. lychees, valued at $3.60 to $8.10/kg). For commercial olive orchards, a rule of thumb is to allow for 8 ML/ha/year including rainfall (Holt and Bicknell 1998). Another method of estimating irrigation water requirements is to supply water at 0.8 × the evaporation rate. The evaporation rate could be estimated for a particular site under consideration, based on data available from the Bureau of Meteorology. For *C. brewsteri*, a requirement of 10 L/tree/day (1.5 ML/ha/yr) for years one to 10, rising to 20 L/tree/day (3.0 ML/ha/yr)

from year 11, was accepted. The cost of water and pumping was taken at $45/ML after Hinton (1995). The total cost for irrigation supply was then $68/ha rising to $135/ha at year 11. Irrigation system maintenance was estimated at $50/ha for carob (Curtis *et al.* 1998). While the cost of maintenance will vary depending on the type of irrigation system used, the same estimate was used in this analysis.

Fertiliser: $500/ha

Fertiliser type and costs vary depending on the chemical and physical characteristics of the site and the nutrient requirements of the crop. An example of fertiliser requirements for a carob orchard with 100 kg pods/tree is 5 kg/tree ammonium sulphate, 2 kg/tree calcium phosphate and 2 kg/tree potassium phosphate (Esbenshade and Wilson 1986). Curtis *et al.* (1998) estimate fertiliser costs at $698/ha for carob (no breakdown of costs given). The fertiliser requirements for *C. brewsteri* will require further study before efficient regimes can be established for individual sites. A figure of $200/ha/yr for years two to nine and $500/ha/yr from year 10 was used in the financial analysis, on the basis that the bulk of the fruit mass will be left on site, reducing nutrient losses. Application costs are considered along with harvesting in the financial analysis.

Weed control: $50/ha

Weed control is estimated at $79/ha for carob (Curtis *et al.* 1998) and $54.60/ha for lychees (Hinton 1995). In the current financial analysis, a lower value of $50/ha/yr from year two is adopted, as light grazing would provide effective weed control. Herbicide should only be required in small amounts to allow easy access for pruning and harvesting.

Insect control: $100/ha

Hinton (1995) estimates that endosulphan treatment for insect control in lychees would cost $0.1029/tree (labour costs not specified). If this cost were doubled to account for price increases, the cost for 417 *C. brewsteri* trees would be $85.84. Chemical and application costs are estimated at $100/ha/yr from year 10. For a more accurate estimate of costs, trials should be carried out under orchard conditions with particular attention given to *Caryedon serratus*, the main pest identified in this study (Chapter 5).

Pruning: $20/ha

Pruning is essential to allow mechanical harvesting in carob, where a clear 1.0 to 1.2 m of stem above ground level is required. The centre of the tree should also be removed periodically to allow light into the tree (Gebhardt 1996). Olive trees are usually staked until they are three years old and trained into a single trunk from 0.8 to 1.2 m high to allow mechanical harvesting (Holt and Bicknell 1998). Pruning costs for carob are estimated $20/ha/yr from year seven (Race *et al.* 1999). The rate of $20/ha/yr from year 8 is applied here (although this scenario includes twice as many trees, the trees will be approximately half the size). Allowance for a heavy pruning ($40/ha) is made for the first pruning, at year 7.

Harvesting: $754/ha

It is likely that conventional fruit and nut harvesters could be adapted for *C. brewsteri* pods. Harvesting machines can be used for carob pods, which have a brittle abscission zone that is easily broken by shaking. In carob, this feature has been accentuated through careful selection of varieties, and this strategy should be possible with *C. brewsteri*. Examination of wild *C. brewsteri* trees has shown that shaking by hand is often enough to dislodge most of the pods if the pods are sufficiently ripe. This stage is usually reached one to two months after ripening.

Contract mechanical harvesting for carob is estimated at $754/ha/yr (including fertiliser and fertiliser application) (Curtis *et al.* 1998). In the present analysis of *C. brewsteri* cropping, the cost of fertiliser is treated separately but its application is included with harvesting. However, the full $754/ha is adapted here because harvesting of *C. brewsteri* would probably require a threshing process on site to separate the seed from the pod on-site, imposing an extra cost at this stage which may not be balanced by saving the cost of transporting material with no value.

Quantitative measures of commercial viability

The net financial returns predicted for a *Cassia brewsteri* orchard were calculated in a spreadsheet (MS Excel) on the basis of discounted cash flow analysis and the projected stream of costs and returns over a 30 yr planning period (summarised in Table 8.2). For this analysis, future cashflows have been discounted to a current value using an interest rate of 7% and this represents the minimum return required by a grower. Five measures were used to assess the potential financial performance of the crop.

1. The Net Present Value (NPV) represents the cumulative total of costs and returns in any future year discounted back to its present value. The PV (Present Value) of a future sum (S_n) at the end of n years with an interest rate of i is calculated as:

   $$PV = S_n \div (1+i)^n$$

   The PV and NPV of a series of unequal yearly costs or returns (A) is calculated using the equations (from Rae 1977, Makeham and Malcolm 1981):

   $$PV_n = A_0 + A_1 \div (1+i)^1 + A_2 \div (1+i)^2 + A_3 \div (1+i)^3 + \ldots + A_n \div (1+i)^n$$

   $$NPV = PV_{returns} - PV_{costs}$$

2. The Internal Rate of Return (IRR) represents the percentage return that an investment is capable of generating over a stated period. It is the rate of interest used in calculating present values that makes NPV = zero (Rae 1977). The IRR was calculated in the spreadsheet using the 'Goal Seek' tool to change the interest rate such that the NPV at year 30 = 0.

3. The benefit/cost ratio is derived by dividing the PV of total returns by the PV of total costs. A benefit/cost ratio of greater than one indicates a profitable venture (Makeham and Malcolm 1981).

4. The payback period is the time taken to repay the capital costs of investment. It can be determined from the first year in which the cumulative NPV is positive (Rae 1977).

5. Gross margins can be used for a direct comparison between crops where there is an enterprise that can be run using existing resources. The gross margin (returns minus variable costs) does not take into account overhead costs such as rates, electricity, insurance and interest.

Table 8.2. Summary of estimated costs and returns for C*assia brewsteri.*

Costs and returns per ha	Amount ($/ha)	Year(s) applied
Costs		
Land value	0	
Survey and design	50	year 1
Land preparation	120	year 1
Installation of irrigation	5000	year 1
Fencing	205	year 1
Growing stock	417	year 1
Total establishment cost	5792	year 1
Irrigation water	68	year 1-10 then 135 from year 11
Irrigation maintenance	50	each year
Fertiliser	100	years 2-9 then 500 from year 10
Weed control	50	years 2 +
Pruning	40	year 7 then 20 in years 8 +
Insect control	100	year 10 +
Insurance	0	
Harvesting + fertiliser application	754	year 11 +
Transport	0	
Returns		
At $1.60/kg seed	1400 from year 11 to 3733 from year 16	
At $2.00/kg seed	1750 from year 11 to 4667 from year 16	
At $2.40/kg seed	2100 from year 11 to 5600 from year 16	
Other products	0	
Light grazing	0	

Results and discussion

The financial model generated the following predicted returns per hectare for a 30 yr planning period using the financial model: NPV $331, IRR 7.2%, benefit/cost ratio 1.020, payback period 29 yr and gross margin from year 16 onwards $2123. The most sensitive cost in the financial model is seed price, a 10% change in seed price changes NPV by 515%. If seed value is increased by 50% (from $1.60/kg to $2.40/kg) IRR increases from 7.2% to 11.5%, NPV from $331/ha to $8661/ha, benefit/cost ratio from 1.020 to 1.530 and gross margin from $2123/ha to $3990/ha, and the payback period is reduced from 29 to 19 yr (Table 8.3). The next most sensitive costs are installation of irrigation, harvesting costs and fertiliser costs. A 10% change in each of these costs results in a change of 151%, 123% and 108% respectively in NPV (Fig. 8.1). Harvesting costs and fertiliser requirements are still relatively poorly understood for the crop and require further study.

Table 8.3. Summary of discounted cash flow analysis (at 7%) for a *Cassia brewsteri* orchard over a 30 year planning period with optimum yield attained at year 16. (Seed prices of $1.60, $2.00 and $2.40 per kg).

Financial measure	$1.60/kg	$2.00/kg	$2.40/kg
NPV ($/ha)	331	4597	8661
IRR (%)	7.2	9.6	11.5
Benefit/cost ratio	1.020	1.275	1.530
Payback period (yr)	29	22	19
Gross margin ($/ha from year 16)	2123	3057	3990

The price of growing stock could also be the most significant cost if grafted trees were required (at $8.00/tree), the establishment costs would increase greatly (from $417/ha to $3336/ha). Even allowing for a reduced planting density of 296 trees/ha the growing stock would cost $2368/ha. This would reduce NPV at year 30 to -$1620/ha and IRR to 6.0%. Any cost increase is more significant early in the projection, hence NPV is particularly sensitive to establishment costs.

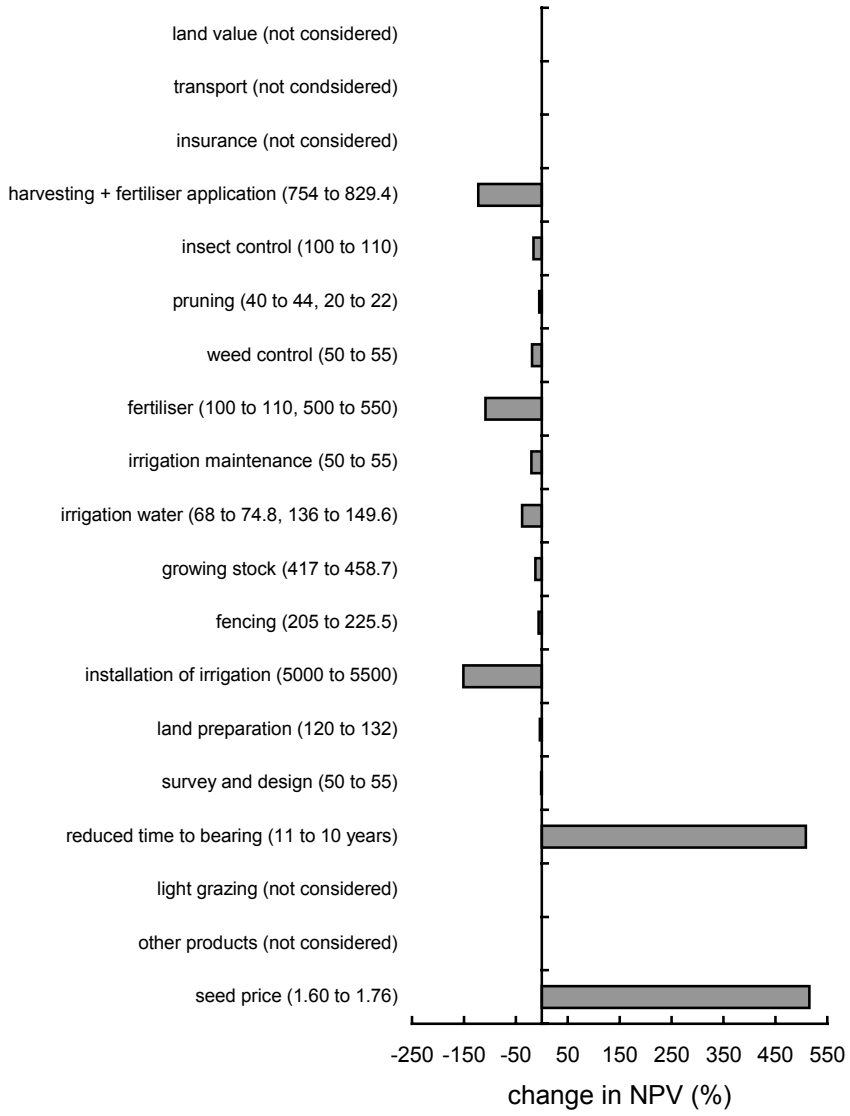

Figure 8.1. Sensitivity of NPV of a *Cassia brewsteri* orchard to changes in costs and returns. (seed price $1.60/kg, 10% increase in each individual cost and return described in Table 8.2).

The cost of superior growing stock may be recouped through reduced time to bearing resulting in earlier returns. If the predicted yields are shifted back one year earlier to commence in year ten, the NPV increases from $331/ha to $2016/ha (Fig. 8.1). An important aspect of perennial fruit crop management is the reduction in the juvenile phase through vegetative propagation and canopy management. While grafted planting stock may appear more expensive than cuttings or seedlings, the effect of earlier returns could outweigh the initial costs over the longer term.

A simple sensitivity analysis such as this is limited in that the results depend on the set of starting estimates for each cost and yield. If the set of assumptions were to change, the relative importance of each component would be different.

General conclusions

The cost of a processing facility capable of processing 1500 t carob seed/year was estimated at $2 million (Curtis *et al.* 1998). A processing facility could be adjusted to process various seed gums, e.g. *C. brewsteri*, carob and possibly guar. A possible advantage of establishing various crops could be consistency of supply, with compensating variation in production of the various crops in the long term. Carob grown in Australia produces ripe pods in late summer (Esbenshade and Wilson 1986), the same time as *C. brewsteri* (Chapter 3). However, both types of seed can be stored for long periods, allowing milling equipment to be used throughout the year. The Australian pet food seed gum market of c. 1200 t/yr could be catered for by a single gum processing facility. To fully utilise this processing facility with *Cassia brewsteri* seed alone would require a total orchard area of 645 ha of *C. brewsteri* supplying 395 t/yr of pet food grade gum from year 11, rising to 1050 t/yr after 16 yr. The estimated establishment cost for this area is $3.7 million in year one. However, with the estimated low IRR over 30 years and a long establishment period before any positive cash flows (12 yr), establishment of *C. brewsteri* orchards cannot be recommended at this time.

Cassia brewsteri is not the first seed gum crop to be considered in Australia. Attempts have been made to establish guar and carob gum industries in Australia since the 1980s. However, guar and carob are not yet grown on a commercial scale in Australia and no seed galactomannans have been manufactured locally. A book on growing carob in Australia (Esbenshade and Wilson 1986) and recent papers (Curtis *et al.* 1998, Race *et al.* 1999) have indicated that the crop should be economically viable in Australia. The investigation of carob as a new crop has received support through RIRDC/LWRRDC/FWPRDC and industry users. Thus far, the largest carob orchard established in Australia consists of only 4500 trees on 40 ha near Burra, South Australia (Esbenshade and Wilson 1986). According to the estimates presented by Race *et al.* (1999), 224,640 irrigated trees (1080 ha) would be required to meet the Australian demand for carob gum. The existing 40 ha orchard would contribute less than 4% of this requirement. Guar has been grown commercially as a seed gum crop in the USA since 1940 (Dea and Morrison 1975). It has been evaluated in Australia since 1980 when the CSIRO began investigating the potential of the crop as a source of industrial gums (Hansen *et al.* 1992).

The establishment period for a new crop industry in Australia is in the order of decades (Fletcher and Kregor 1998), with a longer period to be expected for perennial crops compared to herbaceous annuals. This time frame presents several problems for the establishment of an industry based on *C. brewsteri* gum. There is little scope for recovering the costs of patenting any use of the gum since the term of the patent (20 yr) would expire at about the time significant quantities of product were on the market.

As a tree crop, *Cassia brewsteri* is inherently more difficult to domesticate than an annual plant such as wheat or guar due to the long period from planting to maturity. This not only delays any financial return but makes any breeding program a long-term prospect. On the positive side of the accounts, *C. brewsteri* requires less intensive production practices than a crop such as *Senna tora* or guar, and trees have additional benefits compared to pasture including stock shelter, erosion control and water table (salinity) management. The development of carbon credits may also make tree crops in general more economically viable. Given financial returns for these environmental values, *C. brewsteri* could be a potential crop for land of relatively low value in non-coastal Queensland, where modest irrigation potential exists.

On balance, we advise that investment into production activity of *C. brewsteri* seed gum is risky under present conditions. However, production of this commodity has potential, and reconsideration at a future time is recommended. A pilot planting, perhaps installed as part of a revegetation exercise (e.g. minesite rehabilitation), would be a useful resource from which to base future assessments.

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