Appendix 11

Evaluation of the pollination biology and fecundity of *Calliandra calothyrsus* at Walkamin, North Queensland, Australia

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Abstract

An investigation was made of the reproductive behaviour of a 0.5ha, 4-year old plantation of the fodder tree legume *Calliandra calothyrsus* CPI 115690 (derived directly from the Indonesian land race) managed as a seed crop at Walkamin in north Queensland, Australia, over the period May-October 1998. The main objective of the investigation was to identify reasons for successful earlier seed production at this site in the hope that the knowledge would provide insights into, and hence solutions for, frequent failures in other countries.

In order to estimate components of potential seed yield and sources of loss, records of inflorescence numbers, pod numbers, seeds per pod, and level of andromonoecy were made on randomly selected individual trees. On others, the diurnal course of stigma receptivity was reconstructed through use of the Nile Blue test. A series of pollinator exclusion experiments, both long- and short-term, was conducted, with inflorescences enclosed in cages or pollen proof bags, to allow inferences to be drawn on the nature, activity and effectiveness of pollination agents. These were considered along with observations of the occurrence and behaviour of prospective pollinators to judge the likely contribution of different agents to cross-pollination.

Great variation was recorded, both from tree to tree and between plot-edge and inner trees, in inflorescence and pod numbers per tree, with edge trees having by far the greater average numbers of both. Seed numbers per pod, estimated ovules per pod, and seed set per 100 ovules, were much less variable. Seed numbers per tree thus varied largely with the variation in inflorescence and pod numbers, and averaged 38191 and 7143 per tree respectively for edge and inner trees (equivalent to about 1.71 and 0.32 kg of seed per tree). The plantation as a whole was estimated to have produced 223 kg/ha of seed in the season.

Andromonoecy occurred, varied greatly and largely inexplicably within and between trees and positions in canopy, but overall was not frequent enough (overall average 6 % of observed flowers) to influence seed production materially.

The dominant variable with respect to pollination was the proportion of flowers that set pods rather than the proportion of ovules within an ovary that was fertilised. For example, for edge and inner trees respectively the seed:ovule ratios were 1:1.7 and 1:1.5 while the pod:ovary ratios was 1:50 and 1:25. Stigma receptivity had a marked diurnal rhythm with a peak at about 2000 hours and diminishing receptivity after dawn. Interpretation of exclusion experiments showed that a

significant amount of self-pollination occurred, that pollinators were required to increase pod set and that although insects increased pollination minimally, larger agents did so appreciably. Examination of pollen balls from traps in an adjacent hive entrance confirmed that bees played a negligible part in pollination. Observations showed that Spectacled Flying Foxes (*Pteropus conspicillatus*) were certainly responsible for mass pollen transfer. This, considered with the rhythms of stigma receptivity, left them as prime candidates for the role of most important pollinator. Morning-active flower-feeding birds, notably friar birds and other honeyeaters, could not be eliminated as pollinators, but timing with relation to stigma receptivity, and obvious avoidance of anthers suggests a less important role.

Comparison with published records of seed production of *Calliandra* elsewhere, though difficult because of differences in properties measured, suggests that production from trees at Walkamin was generally better than the best documented in other countries, though not greatly so, and not for any single overriding reason. The high potential seed production at Walkamin is attributable partly to the plantation being in an environment conducive to vigorous reproductive activity of tropical legumes generally, partly to its deliberate management *as a seed crop*, and partly to the timely presence of abundant pollinators in the form of flying foxes. The realisation of that potential in terms of seed recovered is then a matter of harvest method, the preferred one of sweeping fallen seed from hessian spread beneath the trees being highly efficient compared with the usual hand-picking.

Introduction and Rationale

Calliandra calothyrsus is a leguminous Central American tree highly regarded as a provider of fodder, firewood and ground cover in many parts of the world's wet tropics. It was evaluated in Queensland, Australia as a fodder tree, being perceived to be the best of the alternatives to leucaena for use in the many districts where leucaena performs inadequately. This perception led to requests for the tropical pasture seed production team at Walkamin Research Station to develop seed crop management methods, and to provide seed to service the evaluation program. Subsequently, confidence in *C. calothyrsus* as a fodder tree likely to be adopted by Queensland cattlemen declined. Particularly as it was realised that the plant was unproductive in the dry tropics; and as pasture land in the high-rainfall tropics, which are not extensive, moved increasingly over to more lucrative cropping activities such as sugar cane and bananas.

Thus, while the seed production exercise was highly successful, the reason for its existence – at the domestic level – ceased to exist, and plans were made for its cessation. Meanwhile, international interest was aroused in the reasons for the success of seed production at Walkamin, when it failed so often elsewhere. This led Alan Pottinger of the Oxford Forestry Institute to channel funds from the Department of International Development of the UK to allow us to extend the life of the plantation by one season to investigate the reasons for successful seed production at Walkamin. Previous experience of seed production of *C. calothyrsus* in other countries is summarised by Chamberlain and Rajaselvam (1996*a,b*), Rajaselvam *et al* (1996) and Boland and Owour (1996).

The initiative was supported by Joanne Chamberlain (Oxford Forestry Institute), who recognised the potential value of an analysis of the Walkamin situation. She pointed out that "the reasons for this" (i.e. success) "can improve our understanding of the factors controlling seed production in the species, and the results can be applied to other locations where *C. calothyrsus* is an exotic", and suggested specific attention to andromonoecy; breeding system – outcrossing or self-compatible; the activities of pollinating agents, particularly bats; climatic conditions; and the seed source (in fear of material with a narrow genetic base, which is sometimes associated with reproductive inefficiency).

This effectively summarises the rationale for the exercise from an international viewpoint. To this, at the local level, may be added the value of a broader understanding of the reproductive behaviour of tree legumes and the development of effective seed crop management systems which might be harnessed commercially in the event of a seed export market developing.

Background

Climate and geography

Walkamin Research Station lies at lat. 17°08`S, long. 145°26`E at an elevation of 600 m on a lower level of the Atherton Tableland in north-eastern Queensland, Australia. The area is virtually frost-free. Mean daily temperatures for the warmest and coolest months (January and July) are about 25 and 18°C respectively. Rainfall averages about 1000 mm annually, mostly received during the four-month summer wet-season. The dry-season extends normally from April to December, and is reliably dry. The Station is irrigable.

The soil type of the *C. calothyrsus* plantation is a red basalt-derived euchrozem or oxisol derived from late-Tertiary or more recent basaltic flow, deeply weathered, of neutral reaction, and in most respects fertile and easily worked. Under legume cultivation, it needs (and received) P, K and Mo.

Plantation history

The plantation was one of two established locally from a mixture of two lines of seed supplied by Brian Palmer of CSIRO, Townsville, Australia. Both lines had been originally imported from Indonesia under the CPI (Commonwealth Plant Introduction) number 115690 and were of the Indonesian land race. The other plantation, sown at Kairi Research Station 20 km SE of Walkamin and 100 m higher, on a similar soil type but in a markedly cooler, cloudier, wetter climate, grew vigorously but flowered weakly, and was abandoned early for seed production in favour of grazing.

The seed sown at Walkamin had been inoculated with specific inoculant supplied by CSIRO. Inoculation was successful: plants nodulated readily, and no external nitrogen was needed or supplied during the life of the plantation. Seed was directly-drilled in 2-metre spaced rows in January, 1994. Progressive thinning followed obvious overcrowding and consequent suppression of seed production. By the time of the present exercise, rows were 8 m apart and trees mostly spaced at about 2 m along the rows. The population then consisted of 406 trees (12 rows) occupying an effective canopy area of 0.53 ha.

In the first season (1994), plants were not fully grown, flowering and seed production were sparse, and seed was hand-picked. In the second season plant populations were too dense, which suppressed seeding within the crop. By the third and fourth season, satisfactory management

practices had been established. Plants were pruned to about 1.5 m in December of each year. They recovered and grew vegetatively over the wet season to produce a canopy that closed over completely between 8 m rows during early dry season. Inflorescences developed on this crop framework from buds visible by about March, with vigorous flowering from about May onwards. Seed ripened mainly over the September-October period. Hessian was laid on the soil surface beneath the crop canopy, and fallen, mature seed was collected by sweeping over the October-November period. Estimated total productivity was equivalent to 206 and 177 kg/ha of pure, dry seed, with actual recovery of 99 and 85 kg of seed for 1996 and 1997 respectively.

Potential pollinators

Bats have been shown to be the primary pollinators of *C. calothyrsus* elsewhere (Chamberlain and Rajaselvam, 1996; Rasjaselvam *et al* 1996). In the Walkamin district, six species are known to inhabit the area and were considered as potential pollinators of *C. calothyrsus*. Specifically, three species of Australian Fruit Bat (Suborder *Megachiroptera*, Family *Pteropodidae*) are known to feed on blossom/nectar, as an alternative to their usual diet of fruit (Clague and Wybird, pers. comm.); the spectacled (*Pteropus conspicillatus*), the black (*P. alecto*) and the little red (*P. scapularis*). In addition, one tube-nosed bat (*Nyctimene* sp.) and two small blossom bats, the northern blossom bat (*Macroglossus minimus* syn. *M. lagochilus*), and the Queensland blossom bat, *Synconycteris australis* are also known in the area. However the latter is believed not to stray far from rainforest margins, the closest patch being 10 km away. Blossom feeding birds active in the early daylight hours had to be regarded as potentially another group of pollinators. In particular, the honeyeaters include likely candidates.

Insects are not believed to be effective pollinators as they largely avoid contact with anthers and stigmas en route to and from nectaries. Hives of domestic bees had been sited close to the edge of the plantation in every year during the flowering season. This was continued as a precaution until an assessment of their ability to pollinate *C. calothyrsus* could be performed. Little was known of other insects, arthropods, or arboreal marsupials such as glider possums as potential pollinators beyond the observation that the flowering period was the period of least seasonal activity of insects, which are far more abundant during the wet season.

Reproductive biology

Calliandra calothyrsus produces very few pods per tree in comparison to the overall number of flowers that are produced (Chamberlain and Rajaselvam, 1996*a*). A single flower is reported to live for 24 hours, is only slightly protandrous and peak stigma receptivity occurs during the evening. Andromonoecy is a feature of the species and is thought to contribute to low seed production. *Calliandra calothyrsus* is generally thought to be self-incompatible, however a low degree of selfing has been reported (Chamberlain and Rajaselvam, 1996b; Boland and Owour, 1996; Rajaselvam *et al*, 1996).

Figure 1: *Calliandra calothyrsus* at Walkamin, north Queensland, Australia

Materials, Methods and Results

Study details, management and seed collection

Details of history, size, and layout of the Walkamin plantation used for the study have already been given in the Background section. Prior to the experimental period trees were pruned to 1.5 m with a mango trimmer (a disc with a bank of circular saws, angle and height hydraulically controlled, mounted on an arm attached to the front of a heavily-armoured tractor) in December 1997. Re-growth from pruning proceeded vigorously during the subsequent wet season. About 600 mm of rain fell between pruning and the end of May, 1998, when trickle irrigation was installed, and through which a total of about 130 mm of water applied between June and September, 1998.

Experiments relating to the reproductive biology of the Walkamin population were conducted during the flowering season, between May and September 1998. For each row, individual trees were assigned a number and were considered to be either "edge" (located at the end of each row), or "inner" trees (located within a row). The only exceptions were rows 1, 11 and 12 where all trees in these rows were considered to be edge trees. The inner trees were further divided into 5 blocks, each of 63 trees. For each experiment edge and/or inner trees were randomly sampled.

In 1998, due to financial strictures, only part of the plantation was laid with hessian to collect fallen seed. In the remainder of the plantation, once experimental measurements had ceased, inflorescence clusters were reached with the aid of a cherry picker and broken off manually, to be placed on a concrete platform where they dried in the sun and shattered. This produced useful amounts of seed, but did not provide information on potential productivity.

Statistical treatments

With most records collected, estimates of reliability of means and extent of variation are of greater interest than statistically significant differences between treatment means, and therefore the only routinely quoted statistical properties are standard errors (SE) of means. In such cases, it is enough to infer the significance of differences from rules of thumb, taking for example the LSD (Least Significant Difference) between two means at $P<0.05$ as being roughly three times their average SE. Where comparisons between treatment means are critical, simple or factorial analyses of variance have been used as bases for judging differences. For such analyses the statistical unit has been the

individual tree or part of tree, and the value chosen to represent it the mean of an often large and variable number of records.

Estimation of reproductive success

To estimate the reproductive success of the Walkamin population of *C. calothyrsus,* a range of counts were made including whole tree counts of inflorescences and pods, hermaphrodite and staminate floret counts per inflorescence, and seed and aborted seed counts.

Inflorescence and pod numbers: whole tree counts

Methods: In May 1998, five trees were randomly selected from edge plants, and five from inner plants (one tree per block). On each tree all inflorescences greater than 4 cm long were tagged and counted. In July 1998 a second count was performed to account for inflorescences that were immature in May 1998 (<4 cm).

Once tagged, the inflorescences were left to allow natural pollination and pod development. In early July 1998, pods considered to be mature (brown and hard) or intermediate (green and stiff) were harvested. Pod harvest was restricted to edge plants, inner plants lacking harvestable pods. A second, and complete harvest of all pods was made in late August 1998, which included mature, intermediate and immature pods (small and green). Overall pod number per tree was calculated.

Results: Trees located on the edge of the experimental plot showed substantially greater reproductive output than those situated within the plot (inner) in terms of inflorescences per tree, pods per inflorescence, pods per tree, seeds per pod, and % seed set per pod (Table 1). Specifically, edge trees had twice as many inflorescences per tree, which resulted in nearly five times greater pod numbers, than inner trees. Variation between individual trees was great for both measures. The range of total inflorescence number for edge trees was 170-435 inflorescences per tree, and16-252 inflorescences per tree for inner trees. The individual tree for which 16 inflorescences were recorded failed to produce countable inflorescences (> 4cm), or pods, florets remaining too undeveloped to open. Total pod number for edge trees ranged from 3,294 pods to 11,279, and for inner trees, from 866 to 2,251 pods. Differences between edge and inner were statistically significant for all properties except inflorescences per tree, where variation between individual trees concealed positional effects.

Table 1: Total inflorescence, pod and seed number per tree from whole tree counts of trees located at the edge and inside the experimental plot (mean ± s.e.)

Location in plot	Inflorescences per tree	Pods per tree	Seeds per pod	% seed set per pod
Edge	303.40±49.49	6 354.60±1485.54	6.01±0.05	69.78±0.53
Inner	157.40±40.98	1 409.75±704.88	5.07±0.09	58.60±0.92

Seed and aborted seed counts: whole tree counts

Methods: Seed and aborted seed counts were made on pods collected from whole tree harvests. For each harvest, individual pods were run across a light source that illuminated their contents, allowing for efficient counting of the seeds or aborted seeds within. In July 1998, up to 300 pods were counted per tree. In August 1998, due to the large number, and mix of pods harvested, a sub-sample of pods was taken, and seeds counted. Sub-sampling was achieved by thoroughly mixing all pods collected and drawing a representative sub-sample. This sub-sample was further divided into mature pods (seeds counted; useful pods) and immature pods (seeds not counted). The proportion of "useful" pods was determined for each sub-sample and this value used to determine the useful proportion of pods collected from each tree.

Results: The trend for edge trees to be the more vigorously reproductive, measured as inflorescence and pod numbers, was also evident with pod contents, whether measured as seeds per pod or percentage seed set (Table 1). The order of difference, however, was much less, and clearly variation in seed numbers per pod contributed far less to overall variation than did either inflorescence numbers per tree or pods per inflorescence or pod numbers

Floral node, hermaphrodite and staminate floret counts

Methods: A total of 15 edge, and 20 inner trees (four trees per block) were randomly selected. Two developed inflorescences were collected per tree; one from the lower canopy position and one from the upper canopy in late May, 1998. Florets were counted at each floral node. Counts ceased when individual florets were no longer distinguishable, instead only the number of floral nodes recorded until these too became indistinguishable. At ten floral nodes, commencing at the base, 100-300 florets including open flowers and buds, were dissected and the number of hermaphrodite and staminate florets recorded. Flowers were classified as staminate according to Chamberlain (in press) if the gynoecium was reduced, or withered and brown in colour. The percentage andromonoecy per tree was calculated using these figures.

Results: Floret numbers per inflorescence showed a similar pattern to those of other floral counts, edge trees producing more florets per inflorescence than inner trees (Table 2). Differences between positions were statistically undetectable against the background of differences between individual trees. Numbers of visible floral nodes per inflorescence were comparatively consistent, the slightly higher value for edge trees again not being demonstrable statistically. The number of florets per node also did not vary significantly with position, though of itself was a highly variable value, with as many as 50 florets per node counted on some occasions.

The records of percentage andromonoecy showed a curious, inexplicable, but statistically convincing pattern of differences. A factorial analysis of variance revealed a highly significant interaction ($P<0.001$) that reflected higher levels of andromonoecy in the lower levels of the canopy than the upper in edge trees, and the opposite in inner trees.

In a few cases (individual inflorescences), the number of staminate flowers was very high, for example, the percentage andromonoecy for one inflorescence from the lower canopy of an edge tree was 42.5%, and 27.9% andromonoecy was recorded for an inflorescence from the upper canopy of an inner tree.

Table 2: Summary of inflorescence details for edge and inner trees at two canopy positions (mean ± s.e.)

Location in plot	Position of inflorescence in canopy	Floral nodes per inflorescence	Florets per node	Florets per inflorescence	% andromonoecy per inflorescence
Edge	Lower	22.64±0.84	23.49±0.68	532.64±49.37	8.56±3.51
	Upper	22.73±0.66	23.56±0.65	516.36±42.48	4.14±1.08
	Overall	22.68±0.51	23.53±0.47	524.50±31.83	6.35±1.85
Inner	Lower	19.20±0.80	23.53±0.81	451.80±40.14	2.46±1.02
	Upper	20.80±0.88	22.87±0.86	475.60±63.50	6.98±2.46
	Overall	20.00±0.61	23.19±0.60	463.70±36.66	4.72±1.39

Estimation of seed yield

The foregoing records have been summarised to allow estimates of seed production per tree and comparison of the different factors that contribute to it. Average values for each measurement are shown in Table 3. Every factor contributing to seed yield was greater in edge than in inner trees, resulting ultimately in a greater than five fold difference in seed numbers per tree. Beside other sources of apparent waste (or, put another way, routes for potential yield increase), the failure of ovaries to produce pods (of flowers to set fruit) dwarfs all else at 96 to 98%.

As it was not possible to collect all seed from the whole plantation, various attempts were made to estimate crop yield per unit area (kg/ha) from the individual tree records in order to allow comparisons with former years' records. Methods based on separation into edge and inner trees only, and use of the sum of the products of average production and tree numbers of either group were abandoned with the realisation that excessive inter-tree variation introduced massive error. Instead every tree was individually rated by eye with respect to estimated productivity and placed accordingly into one of five ranks. Measured values of seed production were linked to ratings with each of the trees chosen for detailed measurement, and were used to establish a conversion from rank to yield. The relation being non-linear, it was based on simple means for each rank, rather than a regression analysis. The products of estimated yield and number of trees for each rank were then summed to provide an approximate value for overall productivity for the plantation of 118 kg of seed or 223 kg/ha . This confirms that plantation productivity was of a similar order to that of 1996 and 1997 (see earlier quoted figures) and that thus, so far as three years' records allows judgement, 1998 crop growth and development was "normal".

Table 3: Reproductive success of edge and inner trees (mean ± s.e.)

	Measurement	Calculation	Average value for one edge tree	Average value for one inner tree
A	Ovules per ovary (based on total seed + aborted seed numbers)	-	8.8±0.05	8.8±0.07
B	Florets per inflorescence	-	524.5±31.8	463.7±36.7
C	Inflorescences per tree	-	303.4±49.6	157.4±41.0
D	Proportion of staminate florets per inflorescence	-	0.06	0.05
E	Seeds per pod	-	6.01±0.05	5.07±0.09
F	Pods per tree	-	6 354.6±1485.5	1 409.8±704.9
G	Pods per inflorescence	F/C	21	9
H	Seeds per tree	E*F	38191.15	7143.00
I	Florets per tree	B*C	159 133.3	72 986.4
J	Staminate florets per tree	I*D	9 548.0	3 649.3
K	Ovaries per tree (hermaphrodite flowers)	I-J	149 585.3	69 337.1
L	Ovules per tree (potential seed set)	A*K	1 313 358.9	607 393.0
M	Seed:Ovules	E/A	1:1.5	1:1.7
N	% success, ovules to seeds	E/A*100	68.3%	57.61%
O	Pods:Ovaries	F/K	1:25	1:50
P	% success, ovaries to pods	F/K*100	4.20%	2.03%

Nile Blue test for time of stigma receptivity

Methods: In late May 1998, 15 edge trees were randomly selected and 15 inflorescences per tree tagged. Before the start of the experiment, in the late afternoon, all open florets were removed. That evening, one inflorescence per tree was harvested per sample time, at ten sampling times during the night and early morning. Harvesting commenced at 5:50 p.m. and finished at 9:00 am the following morning (Table 4).

Stigma receptivity was determined using the cytochemical test Nile Blue which tests for the presence of lipids on the stigma (Owens *et al* 1991, Allen, 1994). Directly after harvesting inflorescences, pistils were removed and placed into Nile Blue sulphate solution for 30 seconds to one minute. Pistils were rinsed twice with 1% acetic acid which had been heated to 35 ^{o}C, each rinse lasted 30 seconds to a minute. Pistils were then placed in distilled water for up to 5 minutes and mounted in glycerol before viewing under a dissecting microscope. The number of stained

stigmas was recorded, and each stigma given a rating for its degree of staining. The rating system comprised of a scale of from 0.5 - 3 for the region of stigma stained (0.5 = very diffuse (morning only), 1 = blue dots, 2 = dots and surrounding area stained, 3 = whole stigma darkly stained) and a scale from 1 – 3 for the intensity of the stain (1 = light, 2 = middle, 3 = strongly stained). The number of pistils viewed for a given time varied from 38 – 80.

In some cases after harvesting, florets were found to be andromonoecious and thus a test could not be performed on these trees. Out of a total of 15 trees, 11 – 14 trees were tested at any one time. Sampling is summarised in (Table 4).

Table 4: Stigma receptivity Nile Blue test for lipids: summary of the number of trees used and number of pistils tested

	Sampling Time (hrs)	Number of trees	Total number of stigmas viewed
1	1750	13	59
2	1910	11	38
3	2020	12	60
4	2200	14	60
5	0030	12	63
6	0250	13	80
7	0500	13	54
8	0600	12	45
9	0715	14	55
10	0900	13	55

Results: The Nile Blue test for stigma receptivity showed stigmas to be receptive between 1750 and 0900 hrs (Table 5), but with strongest signs of receptivity at 2020 hrs, when the intensity of the stain and area of stigma covered by stain was greatest.

Table 5: Summary of the degree of staining of stigmas by Nile Blue recorded over a 16 hour period from 1750 to 0900 hrs (mean±s.e.)

	Time (hrs)	% pistils stained	Intensity of stain	Region stained
1	1750	54.24	1.08±0.05	1.08±0.05
2	1910	63.16	1.82±0.12	1.45±0.14
3	2020	68.33	2.21±0.10	1.92±0.11
4	2200	60.00	1.36±0.12	1.36±0.12
5	0030	69.84	1.46±0.13	1.25±0.75
6	0250	81.25	1.38±0.06	1.85±0.38
7	0500	75.93	1.31±0.13	1.46±0.88
8	0600	51.11	1.25±0.16	1.08±0.85
9	0715	70.91	1.14±0.06	1.04±0.66
10	0900	36.36	0.85±0.12	1.00±0.87

Ratings: region stained: 0.5 = very diffuse (morning only), 1 = blue dots, 2 = dots and surrounding area stained, 3 = whole stigma darkly stained and intensity of the stain: 1 = light, 2 = middle, 3 = strongly stained

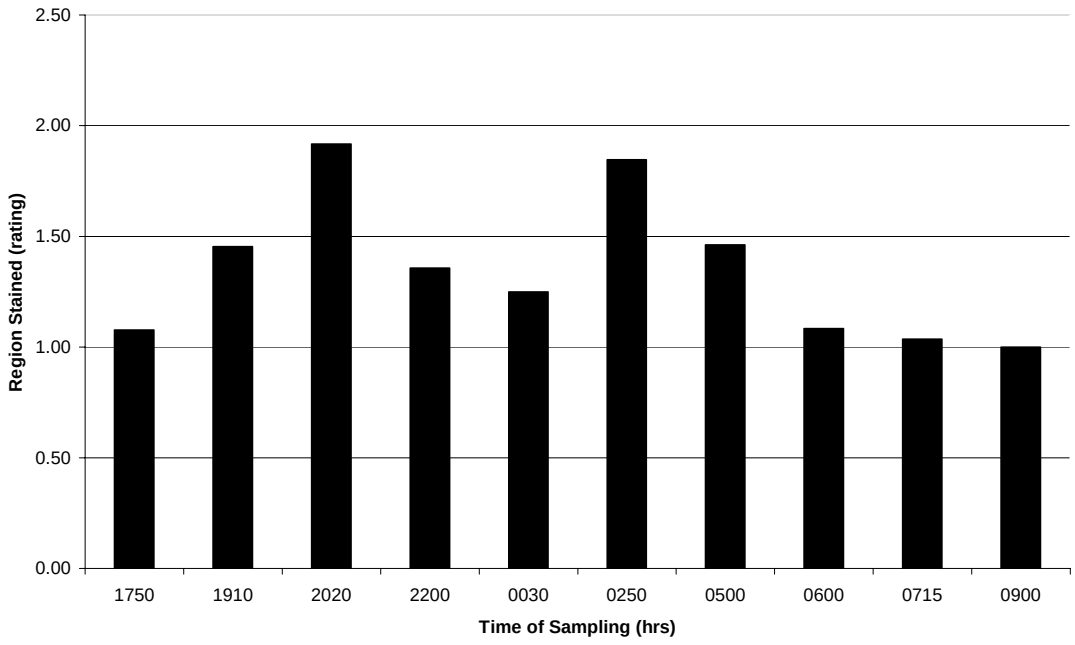

Figure 2: Nile Blue test for stigma receptivity – Rating given for region of stigma stained

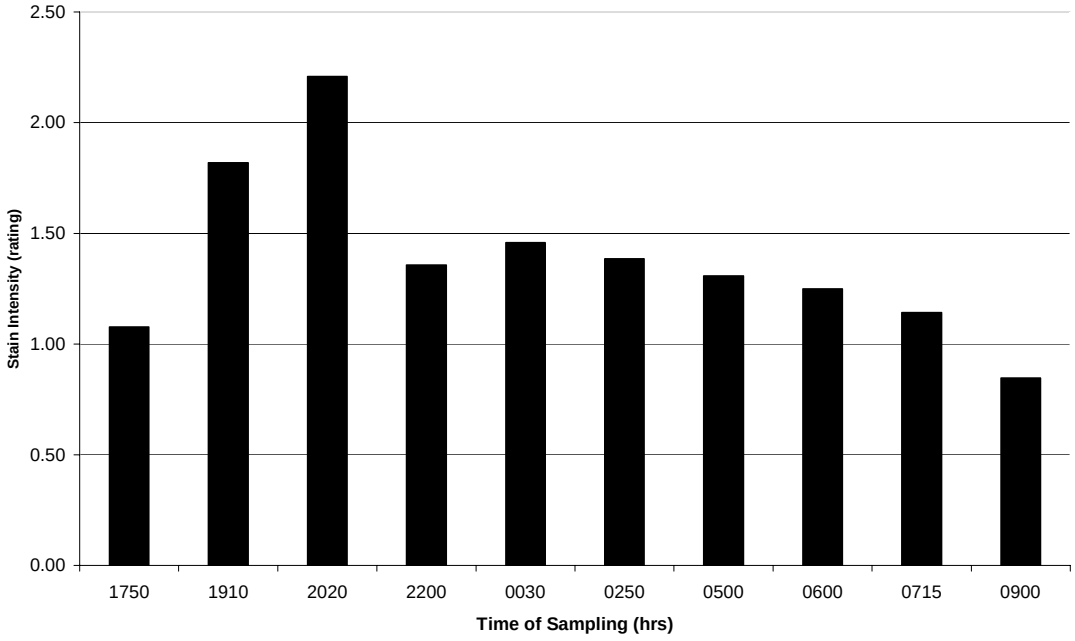

Figure 3: Nile Blue test for stigma receptivity – Rating given for intensity of stain

Pollinator exclusion experiments

To determine the pollinators responsible for pollination of *C. calothyrsus*, experiments were performed to exclude different groups of pollinators. A short-term experiment (five days) was designed to specifically exclude night time pollinators such as nocturnal insects and large bats, and diurnal birds and insects. A long-term exclusion experiment (one month) was also performed which excluded birds and bats or insects, birds and bats. The long-term exclusion experiment was designed to provide a safety net in case there was no pollination during the five days of the short-term experiment. Birds and bats were excluded by use of hand-made bird wire cages, placed over whole inflorescences and squeezed shut at the base of the inflorescence. Insects (and birds/bats) were excluded by enclosure of inflorescences within pollen proof bags made from dressmakers interfacing, bags were sealed with twist-tie at the base of the inflorescence.

Short-term exclusion

Methods: In July 1998, a short-term exclusion experiment (five days) was performed on four edge trees. A total of 60 inflorescences per tree were prepared by removal of all open florets and seed pods (if present). The experiment comprised six treatments, 10 inflorescences per treatment per tree. The treatments are summarised in Table 6. A control of 20 inflorescences per tree was prepared as above and left uncovered for the duration of the experiment (continuously uncovered; CUIP, CUBP).

The experiment began on the evening of the first day just before sunset, when inflorescences were covered with bags (continuously covered insect proof; CIP), and with cages (continuously covered bird/bat proof; CBP) which remained on for five days. Further inflorescences were covered with bags, termed night insect proof (NIP) and with cages, termed night bat proof (NBP). The following morning just before sunrise all NIP and NBP were removed and a new set of inflorescences covered with bags (day insect proof; DIP) or with cages (day bird proof; DBP). This sequence of events was repeated over the experimental period resulting in exclusion of night pollinators over four nights and day pollinators over four days. On the fifth day of the experiment, all bags and cages were removed and pods allowed to develop. After one month pods were harvested and counted, and seed counts performed as described for other monitoring activities.

Table 6: Summary of treatments used in the short-term pollinator exclusion experiments, July 1998

Treatment	Animals excluded	Animals with access	Number of inflorescences per treatment
Continuously covered			
Bag	Insects, birds and bats	None	40
Cage	Birds, bats and mammals	Diurnal and nocturnal insects	40
Covered during day			
Bag	Diurnal insects and birds	Nocturnal insects and bats	40
Cage	Birds	Nocturnal and diurnal insects, and bats	40
Covered during night			
Bag	Nocturnal insects and bats	Diurnal insects and birds	40
Cage	Bats	Diurnal and nocturnal insects, and birds	40
Control	None	All	80

Results: Pod set was recorded on all experimental trees subjected to pollinator exclusion for five days (Table 7). Pod set was highest in the control, differing significantly ($P<0.05$) from all treatments except inflorescences covered with a bag during the day. Analysis of data for which the cover types had been bulked showed pod set in the control to be significantly higher than for night, day or continuous cover. Lowest pod set was recorded on inflorescences enclosed within a cage during the day. Inflorescences continuously covered (bag), or covered during the day with a bag, produced more pods than those covered with a cage, though the differences were only marginally significant statistically at $P<0.05$. Much greater differences occurred between individual trees in pod and seed set than between treatments.

Records of seeds per pod are confusing to interpret. Measured simply as seed numbers per pod (Table 8), they present a general uniformity, with only small and insignificant differences between types of cover and the times when they were applied. Expressed as seeds produced as a percentage of ovules, however, they reveal an anomalously low average values for inflorescences covered with a cage or a bag during the day (Table 9) and an anomalously high one for inflorescences

continuously covered with a cage. These records are difficult to reconcile with those of seeds per pod and are essentially uninterpretable.

Table 7: Pod set per inflorescence following pollinator exclusion over a five-day period, using bags and cages (mean±s.e.)

	Cover	Night	Day	Continuous	Combined
	Cage	1.23±0.20	0.58±0.17	0.82±0.19	0.87±0.13
	Bag	1.28±0.25	2.26±0.60	1.74±0.38	1.75±0.25
Combined		1.25±0.19	1.41±0.32	1.27±0.22	
Control	None	-	-	3.38±0.68	-

Table 8: Seeds per pod following pollinator exclusion over a five-day period (mean±s.e.)

	Cover	Night	Day	Continuous	Combined
	Cage	5.35±0.36	5.06±0.59	5.91±0.44	5.51±0.25
	Bag	6.00±0.36	5.99±0.17	5.32±0.33	5.77±0.18
Combined		5.7±0.26	5.82±0.24	5.53±0.26	
Control	None	-	-	5.74±0.14	

Table 9: Percentage seed set per pod following pollinator exclusion over a five-day period (mean±s.e.)

	Cover	Night (%)	Day (%)	Continuous (%)	Combined (%)
	Cage	61.29±3.03	58.50±4.82	72.27±3.82	65.9±1.85
	Bag	69.17±3.30	66.85±2.65	63.39±3.20	66.3±1.74
Combined		65.57±2.29	65.53±2.35	66.58±2.49	
Control	None	-	-	68.04±1.31	

Long-term exclusion

Methods: This experiment was performed concurrently with the short-term exclusion experiment, although different trees were used. Forty inflorescences per tree, on each of four edge trees were prepared by removal of all open florets and seed pods (if present). The experiment comprised two treatments, 10 inflorescences per treatment per tree (Table 10). Bags or cages were placed over inflorescences. A further 20 inflorescences per tree were left uncovered to act as the control. After

one month the bags and cages were removed and resulting pods harvested and counted. Seeds and aborted seeds were recorded as described earlier.

Table 10: Summary of treatments used in the short-term pollinator exclusion experiments, July 1998

Treatment	Animals excluded	Animals with access	Number of inflorescences per treatment
Continuously covered: bag	Diurnal and nocturnal insect, birds and bats	None	40
Continuously covered: cage	Birds and bats	Diurnal and nocturnal insects	40
Control	None	All	80

Results: Pod set was recorded on all trees, for all treatments (Table 11). The overall pattern was similar to results obtained after short-term exclusion. Specifically, pod set differed significantly between treatments ($P<0.05$), highest pod set recorded for the control (no cover) and the least pod set recorded on inflorescences covered by a cage.

The number of seeds per pod (Table 12) and percentage seed set (Table 13) did not differ significantly between treatments. Average seed numbers set per pod were close to six, and between 64 –67% seed set was recorded. Trees differed significantly ($P<0.05$).

Table 11: Pod set per inflorescence following pollinator exclusion over a one-month period, using bags and cages (mean±s.e.)

Cover	Pod set per inflorescence
None (Control)	30.08±3.43
Cage	6.33±1.30
Bag	17.64±2.32

Table 12: Seeds per pod after pollinator exclusion over a one-month period (mean±s.e.)

Cover	Seeds/Pod
None (Control)	6.23±0.14
Cage	5.94±0.35
Bag	6.19±0.21

Table 13: Percentage seed set per pod after pollinator exclusion over a one-month period (mean±s.e.)

Cover	% seed set
None (Control)	66.60±1.29
Cage	64.52±2.63
Bag	67.65±1.67

When combined effects of short-term treatments are considered beside those of long, some consistency in the trends becomes apparent, with pod set being reduced to about half by enclosure in bags and to about a quarter with use of cages (Table 14).

Table 14: Comparison of the effects of short- and long-term exclusion by cover type on pod set (values as % of control)

	Short-term*	Long-term
Cage	26	21
Bag	52	58

* values combined across night/day/continuous

Observation of birds, bats and insects

Methods: The presence, behaviour and time of visits of each were noted while performing other experiments. Specifically, the presence of birds and bats was noted during the stigma receptivity experiment, which comprised 10 observation times, from 5:50 p.m. to 9 am the following morning. Throughout the flowering season the plantation was visited after dark and the presence and behaviour of bats observed. In addition, early morning visits were made to observe the presence and behaviour of birds. The species seen to be visiting the *C. calothyrsus* population were identified. Mist netting on one evening and one morning was attempted, but without success, as no bats or birds were captured. Early in the season the behaviour of insects was observed and it was concluded that they were unlikely to contribute substantially to pollination of *C. calothyrsus.*

Observations of bats were made with the aid of a 12 V spotlight covered with red cellophane. The light source was largely ignored by flying foxes, and when care was taken to avoid noise or rapid movement, behaviour could be observed from as little as one metre from them.

Failure to catch wild specimens of bats, coupled with the need for positive evidence of pollen transfer, led us to borrow a pair of captive Queensland Blossom Bats (there being no Northern Blossom Bats available in captivity) and a single tame Spectacled Flying Fox. The Blossom Bats were placed in a temporary cage in the form of a mosquito net draped over a flowering C*alliandra* branch, but they showed little tendency to feed, possibly through sensitivity to the disturbance. The fox, on the other hand, was unperturbed by human company and entirely obliging in its willingness to feed.

Bats

Over the main flowering period, Spectacled Flying Foxes (*Pteropus conspicillatus*) commonly visited the *Calliandra* plot, arriving just after dark and remaining until the early hours of the morning (Table 15). Counts of arriving flying foxes made on two occasions, provided estimates of a little over 30 individuals. Flying foxes were not present every night, and later in the flowering season visits were much reduced both in number of bats and frequency of visits.

Table 15: The presence of birds and bats at Walkamin from 1750 to 0900 hrs in May 1998

	Time (hrs)	Pollinator observed
1	1750	*Philemon corniculatus* (Noisy Friarbird)
2	1910	*Pteropus conspicillatus* (Spectacled Flying Fox)
3	2020	*P. conspicillatus*
4	2200	*P. conspicillatus*
5	0030	*P. conspicillatus*
6	0250	*P. conspicillatus*
7	0500	*P. conspicillatus*
8	0600	*Lichmera indistincta* (Little Brown Honeyeater)
9	0715	*L. indistincta*, *P. corniculatus*
10	0900	*L. indistincta*, *P. corniculatus*

Observations during the night, showed *P. conspicillatus* to feed almost continuously on nectar which could been seen glistening on inflorescences. Their habit was to crash into the upper branches, climb down the branch, and approach the inflorescences from the *inside, on foot,* as it were. For movement within a tree canopy, they adeptly used the hook on either wrist, combined with their hind legs for locomotion, the wings tucked back against the body. A single inflorescence was worked in a matter of seconds, and foxes were observed to move continuously from inflorescence to

inflorescence and would fly from one tree to another, sometimes of their own volition, but more commonly when harried by another fox.

Their feeding habit was to place their heads into the mass of anthers and stigmas of an inflorescence, and lick the nectar from the base of the flowers with quick movements of the tongue. As a result their faces and chests came into constant contact with floral parts and broken anthers were often seen on their fur. Recently visited inflorescences were left with an untidy, disordered, bent look.

The feeding behaviour of the tame fox was essentially the same as that of the wild foxes, though it was conspicuously less agile and its movement was constrained by its unwillingness to release its grip on the belt of its keeper. Nevertheless it fed equally voraciously on nectar, and rapidly accumulated pollen on both its face and breast, confirming the potential of the species to transfer large amounts of pollen between trees.

The role of the Blossom Bats remains uncertain. The Northern Blossom Bat was certainly recorded in the plantation by Clague and Whybird[1] in very small numbers, and it is reasonable to infer from its presence that it was feeding. But even when its inconspicuousness is taken into account, it seems unlikely to have been present in the plantation in sufficient numbers and sufficiently long to have more than a fraction of the pollination potential of the Spectacled Flying Fox. The failure of the captive blossom bats to feed means nothing, partly because of the circumstances in which they were introduced to inflorescences, partly because they were Queensland Blossom Bats which, while virtually identical in appearance to the Northern Blossom Bats, are thought not to stray far enough from the rainforest margins to be common at Walkamin.

It was concluded from these observations that Spectacled Flying Foxes were potentially responsible for much intra- and inter-tree dispersal of pollen. Although the exact number of inflorescences visited on a given night is unknown, their behaviour, numbers, and the duration spent in the *Calliandra* population suggest that it would be high.

Birds

Six species of honeyeater were observed at various times to be present in the *Calliandra* (Table 15). Four brown honeyeaters, *Lichmera indistincta*, (probably, in fact, four pairs) maintained distinct territories within or including some part of the plantation, made obvious by consistently located dawn calling over the whole flowering period. Relatively large numbers of the noisy, *Philemon cornicuatus* and little friarbirds, *P. citreogularis*, 30 or more at a time, visited the plantation soon

after dawn over the same period and worked conspicuously over inflorescences. Smaller family groups of blue-faced honeyeaters, *Entomyzon cyanotis,* behaved similarly, though remaining for shorter times each day. Yellow (*Lichenostomus flavus*) and white-throated (*Melithreptus albogularis*) honeyeaters appeared occasionally and singly.

Birds of other groups present were pale-headed rosellas (which ate and destroyed flowers) and various predatory or insectivorous species unlikely to be significantly involved in pollination (a resident pair of spangled drongos, an itinerant brown goshawk, a rufous whistler, a lemon flycatcher, fantails, various cuckoos, etc.). Surprisingly, rainbow and green lorikeets, though voracious nectar feeders on other tree species and frequently seen flying over the plantation, were never observed to alight in the trees.

Observations of numbers, time spent in the *Calliandra,* and behaviour of birds led us to the opinion that only the friar birds could have made a serious contribution to pollination. Even they, however, went to great lengths to avoid contact with anthers. They carefully angled their long curving bills and narrow heads in what appeared to be a conscious attempt to avoid contact with the rest of the flower, while extracting the nectar with their tongues. Consequently we have concluded that, while we cannot exclude the possibility of birds as agents of pollen transfer, they are probably relatively unimportant compared with flying foxes. As no birds were caught in the attempt at netting, no direct evidence was obtained about whether or not pollen was being transferred by birds.

Arthropods

The flowering period of *Calliandra* in north Queensland is at a time of relatively low insect activity. Nevertheless ants, small moths, spiders, and a range of less conspicuous arthropods were present at all times. Neither by night nor day did they give an impression of being serious pollinators, and insects and moths caught by nets lacked polyads on their bodies.

Beehives had been placed beside the plantation in every previous season, and four were present throughout the flowering period in 1998, one fitted with a pollen trap. Bees worked *Calliandra* flowers conspicuously and virtually constantly in all suitable weather, but they clearly avoided anthers where possible (sometimes strong wind made it impossible). *Calliandra* pollen, readily identifiable from its characteristic polyads, occurred in the pollen balls collected in the trap at so low a frequency as to be close to the lower limit of detectability. It was therefore concluded that

conventional wisdom was indeed correct and that bees were not significant pollinators of *Calliandra.*

Arboreal mammals other than bats

Rats, probably the common endemic canefield rat, *Rattus sordidus*, entered the Kairi *Calliandra* plantation in quantity and stripped bark destructively but neither they nor evidence of their presence was observed at Walkamin. Neither they nor the feral cat, the only other arboreal placental mammal present, can seriously be regarded as potential pollinators. The only likely potential marsupial pollinator known to be present in the Walkamin district was the feathertail glider (*Acrobates pygmaeus*), a minute possum. These can occur in high numbers and are very active nocturnal flower-feeders, therefore must be considered. However the position of the *Calliandra* plantation such that there was large gap between it and the canopy of the natural eucalypt woodland created by a roadway and a ditch, plus the degraded condition of the adjacent woodland, counted against the likelihood of their presence. In fact no gliders or other arboreal marsupials were ever observed near or in the *Calliandra*.

Discussion

This study was initially intended to explain the cause(s) of what appeared to be an unusually high yield of *Calliandra calothyrsus* seed in a population growing as an exotic at Walkamin, north Queensland, Australia. The approach was to quantify the reproductive output of the population to determine where differences in seed production lay, and to determine the vector(s) responsible for pollination. Results have since shown that the Walkamin population may not differ significantly from other carefully monitored populations in any one aspect of its reproductive biology. Despite this, the exercise raised some interesting findings which point to ways of improving production, shed light on other aspects of *Calliandra* biology, and suggest routes by which failure of seed production, seemingly frequent though seldom documented, might be addressed.

Edge effects

One of the most striking results of the study was the effect of tree position on relative tree reproductive success, trees located at row ends significantly more successful. This was reflected in production of greater numbers of flowers, inflorescences and pods compared to those located within the plot. Edge trees were free standing on three sides, the fourth side abutting the next tree within the row. In contrast, inner trees were in close contact with each other, not only with their immediate neighbours, but also with trees of adjacent rows, often their canopies overlapping. Competition, either for light, water or soil nutrients, is the most likely explanation for these differences, as the management practices were the same for all trees. Progressive thinning over the last four years has resulted in the current spacing of 2 m gaps between trees within a row, and 8 m gaps between rows. The results from this study indicate these spacings are too close to promote maximum seed production and that further separation of individual trees is required. The ultimate aim would be to make trees within a row behave like edge trees, a change that would probably substantially increase productivity per tree and per unit area. If, for example, a spacing between trees within rows of 4 m achieved this objective, then seed production would be raised from the present value of about 200 kg/ha to something over 500.

Pod set

Of all the variables recorded in the analysis of reproductive success, the one most striking when one looks for ways of increasing seed numbers per tree is the very low rate of pod set (the number of pods produced expressed as a percentage of the number of ovaries). Relatively small absolute gains

over the measured values of 4.2% and 2.0% for edge and inner trees respectively could obviously translate into considerable increases in seed production. The edge effect shows that pod set is under the influence of factors sensitive to spacing as well as of the more direct pollination variables, and other variation suggests other undefined environmental or genetic influences. It is possible, of course, that the branch's capability to supply assimilate or redistribute mineral nutrients limits the number of pods that can successfully form. Damage to flowers and/or ovaries may also be a factor reducing set. Flying foxes may exact a price for pollination in the form of damage – certainly the appearance of an inflorescence that has been visited by a flying fox, suggests it. Birds of at least one species – the pale-headed rosella – have been observed to graze on flowers. The debris of blown and aborted flowers that hangs from inflorescences is normally infested with the larvae of lepidopterous insects that may feed on living as well as dead flowers. Fungal flower blights such as Botrytis and Anthracnose are commonplace and highly destructive on flowers of trees of several other exotic species in districts where *Calliandra* is grown. There is clearly scope for investigation of factors other than pollination that lead to success or failure of pod set.

Seed numbers per pod

Seed number per pod was the only variable for which there was little or no variation between trees or treatments. It seemed that the critical factor in successful pollination were the events leading to pollen and stigma contact, and that once a polyad reached the stigmatic cup, the resultant seed number was fairly constant. Thus seed number per pod provided the least useful information of differences within the population, pod number being a much more informative measure.

Genetic variation

Significant variation between trees was recorded for all variables measured except seed numbers per pod. Although it is outside the scope of this exercise to try to discriminate between genetic and environmental effects, it is impossible not to form the opinion that there was considerable genetic variation within the population of trees in the plantation, and that it extended to details of fecundity. Apparent genetic variation had been noticed in past seasons in properties such as flowering time, flowering intensity (subjectively observed), pod shape, size and colour, etc. It is germane to record this in view of the doubts that have been expressed (Joanne Chamberlain[2], pers. comm.) about the narrow genetic base of the Indonesian land race from which the material was derived. It is also worthy of note in the same context that both we (at Kairi) and Brian Palmer[3] (at Lansdown) have encountered occasional plants of a white-flowered contaminant not *C. calothyrsus*, presumably

introduced with the seed. This raises possibilities of contamination and cross-pollination with other introductions in the nurseries in Indonesia where the original seed was collected.

Comparison with reproductive success in other documented populations

The reproductive success of edge and inner trees is compared with that of *C. calothyrsus* recorded in three other countries where it is grown in Table 16. The comparison has led us to the conclusion that, with the exception of some aspects of edge trees, such as inflorescence number, the reproductive success of *C. calothyrsus* at Walkamin overall was slightly higher, but not greatly so, than plantations elsewhere. For example, the number of floral buds per node and number of nodes per inflorescence were comparable to those reported in Kenya (Boland and Owour, 1996), and when comparing inner trees with other populations there was no difference between total number of inflorescences per tree. There is a risk, of course, that comparison with published records of carefully managed trees distorts the general picture that comes from anecdotal evidence of frequent but unrecorded failure of seed production.

Andromonoecy

One aspect thought to contribute to the low seed yield observed in *C. calothyrsus* populations in areas such as Kenya (Boland and Owour, 1996) and Honduras (Chamberlain, in press), is a syndrome called andromonoecy. This syndrome is represented by inflorescences with a high proportion of staminate (male only) flowers. At Walkamin, andromonoecy represented only a slight reduction in the overall number of ovaries present in the population, the mean proportion of staminate flowers and buds being 0.06. A similar value (0.045) was recorded by Chamberlain (in press) in 1994 for unpollinated flowers in Honduras. However, other values of andromonoecy reported in Chamberlain's paper were much higher. For example, the proportion of staminate flowers for unpollinated flowers in 1995 was 0.118 and for buds it was 0.194. Both values at least double those reported at Walkamin. Andromonoecy was also reported in Kenya, though no mean values were given, which made comparison difficult (Boland and Owour, 1996). These authors inferred that the degree of andromonoecy was significant, suggesting that although their range included values of 0.02 staminate flowers per inflorescence, values were generally higher (up to 0.7). In comparison, at Walkamin, high values (>0.1) were the exception, rather than the rule.

Table 16: Summary table comparing floral, fructal and breeding system characteristics reported for *C. calothyrsus* growing in different countries

Measurement	Walkamin Edge trees	Walkamin Inner trees	Sri Lanka (Rajaselvam *et al* 1996)	Kenya (Boland & Owour 1996)	Honduras (Chamberlain, in press)
Floral characteristics					
Floral buds/node	23.5	23.2	-	24 (18.29)	-
Nodes/inflorescence	22.7	20.0	-	13-19	-
Floral buds/inflorescence	524.5	463.7	-	304 (123-516)	-
Inflorescences/tree	303.4	157.4	-	128	-
Ovules per ovary	8.8	8.8	-	12	-
Polyads/flower	-	-	-	296	-
Ovule:pollen	-	-	-	1:25	-
Prop. staminate flowers and buds/inflorescence	0.06	0.05	-	0.05-0.5 (b/w trees) 0.02-0.7 (b/w inflor.)	0.143 (node 1) 0.566 (node 14)
Prop. staminate flowers/ inflorescences (unpollinated)	-	-	-	-	0.045 (1994) 0.118 (1995)
Prop. staminate flowers/inflorescences (pollinated)	-	-	-	-	0.153 (1994) 0.277 (1995)
Prop. staminate buds/inflorescence (unpollinated)	-	-	-	-	0.194 (1995)
Prop. staminate buds/inflorescence (pollinated)	-	-	-	-	0.387 (1995)
Fructal characteristics (natural poll'n)					
% fruit set (ovaries to pods)	4.2 %	2.03 %		2.05 %	7.54 %
Pods/inflorescence	21	9	-	23	4.66
Seeds/100 pods	601	507	453-617	-	-
Aborted seeds/100 pods	255	369	50-142	-	-
Pods/tree	6354.6	1409.8	-	-	-
Breeding system					
% pod set/inflorescence: self pollination	-	-	2.6 %	11 and 12.9% (2 expts)	-
% pod set/inflorescence: cross pollination	-	-	33.3%	7.2 and 30.7 (2 expts)	-
Seed/pod: self pollination	5.77	-	0.7	-	-
Seed/pod: cross pollination	-	-	8.4	-	-

In addition, Chamberlain (in press) reported that as the maternal investment increased, so did the proportion of staminate flowers towards the distal end of the inflorescence. No such relationship was apparent at Walkamin, though controlled manipulation of maternal investment had not been attempted. There was, however, a significant interaction between the proportion of staminate flowers, the location of the tree (edge vs inner), and the position of the inflorescence in the canopy (lower vs upper) at Walkamin. This interaction was the inverse between edge and inner trees, a greater degree of andromonoecy recorded in the lower canopy of edge trees, while a higher degree recorded in the upper canopy of inner trees. Light may be responsible for these differences, particularly for inner trees, which were heavily shaded by neighbours, all except the upper canopy. This result was the opposite of what had previously been thought; Bertin (1982), suggested that greater light allowed inflorescences to produced more hermaphrodite flowers, and thus more fruit set. Instead at Walkamin, inner trees produced more staminate flowers in the upper canopy where light was greatest. There may be another explanation. Pods are mainly concentrated in the upper canopy of inner trees, and if the same relationship exists at Walkamin as was reported by Chamberlain (in press) for plants in Honduras, increased maternal investment i.e. pod production, increased the proportion of staminate flowers produced. However, this can only serve as speculation, as again structured manipulation of maternal investment was not attempted at Walkamin.

Also, if increased maternal investment increases the degree of andromonoecy (Chamberlain, in press), then the low overall proportion of staminate flowers recorded at Walkamin may be directly related to the fact that the population is managed like a seed crop, in which irrigation and fertiliser possibly counteract the affect of limited resources.

Pollination

The vectors responsible for pollination at Walkamin were determined in two ways – by observation of floral visitors, and by the systematic exclusion of these visitors. Before they could be assessed, it was critical that the diurnal rhythm of stigma receptivity be confirmed. For example, other studies have found peak receptivity to occur between early evening and early morning (Rajaselvam *et al*, 1996; Boland and Owour, 1996). In this study a Nile Blue test for lipids confirmed receptivity during the night, though no statistically significant peak of receptivity was recorded, and there were no significant differences between times. Instead there was a lot of variation in staining between stigmas at a given time due to stigmas being of different ages. This range of stigmatic age was

believed to be the result of the experimental procedure, where, although all open flowers were removed prior to experimentation, additional flowers continued to open during the night resulting in samples of mixed stigmatic age. This occurrence would have implications on pollination, as a small proportion of stigmas would have been at peak receptivity when day pollinators arrived. However overall receptivity was greatest at night, confirming the importance of nocturnal pollinators to the pollination of *C. calothyrsus.*

In calliandra's native range in Honduras, nectivorous bats were found to be the main pollinators of *C. calothyrsus*. In particular, bats of the genus *Glossophaga* (Glossophaginae) visited repeatedly (79.6% of all observations), while other insectivorous bats and hawkmoths visited to a lesser degree (Chamberlain and Rajaselvam, 1996). Also, in Sri Lanka where *C. calothyrsus* is planted as an exotic, bats were reported to be important pollinators. Exclusion experiments showed two species of bat, including the Sri Lankan dog-faced bat and a species of Sri Lankan fruit bat visited the *Calliandra* population (Rajaselvam *et al*, 1996).

Bats were also found to be the primary pollinators of *C. calothyrsus* at Walkamin. Specifically, the Spectacled Flying Fox (*Pteropus conspicillatus*), known to roost in a small patch of rainforest 10 km south of Walkamin was frequently observed visiting the plot. This species is primarily a fruit eater, though the nectar source represented by the plantation of *Calliandra* at Walkamin appeared to be sufficient for repeated visitation by this species during the peak flowering period. Their numbers and behaviour whilst in the plantation were such that effective cross-pollination would have been achieved. For example their habit of progressively moving over the tree and consuming nectar, which transferred pollen onto its face and chest would have promoted self pollination, and their movement from tree to tree, would have effected pollination between trees. Observations of the tame flying fox confirmed this behaviour, the tame animal accumulating much pollen on its face and body in such a way that guaranteed contact between pollen and stigmas of subsequent flowers visited. We were unable to catch specimens of the Northern Blossom Bat, though its presence at Walkamin was confirmed by Clague[1] (pers. comm.), and knowledge of its numbers and behaviour would suggest that it is a potential, but not significant pollinator of *Calliandra.*

The presence of birds on *Calliandra* inflorescences has not been reported before. For example, in Sri Lanka, although pollinator observations were carried out over a 24 hour period, birds were not observed (Rajaselvam *et al*, 1996), and in Honduras, as mist netting was confined to the evening no birds were captured (Chamberlain and Rajaselvam, 1996). This was not the case at Walkamin,

many different birds observed to frequent and work the flowers. However, their contribution to pod set at Walkamin was thought to be minimal as suggested by their feeding behaviour, which tended to avoid contact with anthers, and the fact that plants were not at peak stigma receptivity when birds were present. Even so, they should not be overlooked as potential pollinators, as they worked the flowers in the early morning, a time when some stigmas were still receptive.

In addition, the observed behaviour of insects on *Calliandra* flowers did not appear to facilitate pollination when combined with the floral structure, the anthers and stigma being a long way from the nectary. The lack of involvement by insects has also been reported by Rajaselvam *et al* (1996). Bees observed on inflorescences tended to rob flowers of nectar, rather than transfer pollen, and the low frequency of *Calliandra* pollen observed in pollen balls confirmed that bees did not contribute to overall pollination.

The exclusion experiments were designed to systematically exclude pollinator groups, the short term experiment serving to separate night and day pollinators, and bags and cages used to separate pollinator types, bird/bat or insect. In both experiments the control (continuously uncovered) produced the greatest number of pods, confirming that pollinators were necessary to increase pod set at Walkamin. Complete exclusion of birds and bats was not achieved by the cage design owing to its size and weight, which caused the inflorescence to droop, and in some cases, allowed anthers and stigmas to protrude from the cage. In addition, because of the agile nature of bats at Walkamin, and the length of their tongue, it is thought that bats would have had access the flowers regardless (Clague[1], pers. comm.). Despite this, some surprising results were obtained from the exclusion experiments, the least of which was the high pod production due to self-pollination recorded for inflorescences covered with bags (complete pollinator exclusion). Although the percentage fruit set after self-pollination could not be determined as the original number of flowers present was unknown, pod set after enclosure for one month produced almost 20 pods per inflorescence. This value was comparable to average pod set after natural pollination (this study, whole tree counts), and was greater than that reported for natural pollination elsewhere (Chamberlain, in press). Thus when data are interpreted from the exclusion experiments the occurrence of self-pollination must be considered.

There was no difference in pod set between inflorescences enclosed within a cage or bag overnight, which suggested that nocturnal insects with access to caged inflorescences contributed very little to overall pod set (nocturnal insects are relatively scarce in north Queensland at the time of flowering

of *Calliandra*, as it is both dry season and "winter"). This has similarly been shown in Sri Lanka, very little seed set (0.98%) recorded after enclosure within cages (Rajaselvam *et al*, 1996). In contrast, significant differences were recorded in pod set between inflorescences covered during the day; those enclosed within a bag producing significantly more pods than caged inflorescences. The exposure of inflorescences to nocturnal pollinators when stigmas were receptive would have promoted pod set, and the subsequent enclosure during the day would have caused different affects. For example, it is hypothesised that within the bag humidity was high and reproductive parts were in close contact, thus promoting self-pollination to a greater degree than inside the cage, which was much more open. In fact humidity has been shown to promote pollination in other species. These results are further supported by pod set after continuous cover in both short- and long-term experiments. These treatments recorded highest pod set in uncovered inflorescences, then bagged, and caged had the least number of pods. The difference in pod set of bagged-day, versus during the night, may reflect a difference due to a double promotion of pollination – firstly by exposure to pollinators at night when the stigmas are receptive, and secondly as a result of the conducive environment for self-pollination provided by the bag.

Although detailed conclusions cannot be drawn, three general conclusions can; that pollinators appear to promote pod set, that a reasonable degree of self-pollination is possible, and that pod set is greatest when inflorescences are exposed at night when receptivity is highest.

Similar to studies in Kenya (Boland and Owour, 1996), sporadic pod set was observed on floral spikes at Walkamin, small regions bristling with pods, while others lacked pods completely. Sporadic pollinator visitation was suggested as the cause of this pattern in Kenya (Boland and Owour, 1996), and is the favored explanation at Walkamin. Specifically, flying foxes were observed in reasonable numbers on some nights, and completely absent, or reduced, on others. This behaviour combined with the possibility that not all trees within the population were visited on a given night may cause this pattern of pod set.

Sporadic pod set was also observed in the long- and short-term exclusion experiments. Initially there was some concern that the duration of the short-term experiment was insufficient to ensure pollinator visitation to all experimental trees. However, results showed that all trees were visited, but to different degrees, significant differences recorded in pod set between trees and between inflorescences of an individual tree. One cannot rule out the possibility that other factors influenced pod set, but as some inflorescences within a tree had high pod set, while others from the same tree

had low, or no pod set, it appeared that visitation may have been the limiting factor. Variation in pod set was less for the long-term experiment, all trees and inflorescences having reasonable numbers of pods suggesting that all trees were visited.

Overall it appears that suitable cross-pollinators are present in the form of the Spectacled Flying Fox at Walkamin. These animals are not present continuously throughout the flowering season, most likely other food sources, such as ripening fruit taking precedence. The species is also believed to be nomadic, favouring the coast in cold weather. The winter of 1998 was unusually warm on the Atherton Tableland, and perhaps for this reason their absence, at least from daytime roosts in the nearby Tolga Scrub, was of no more than a week's duration. An apparent contributor to overall pod set was the potential of trees to self-pollinate, a factor which was likely to have been promoted by flying fox visitation. The population displayed a similar reproductive pattern to populations reported elsewhere, stigma receptivity commencing in the evening and continuing until the morning. Continued but reduced receptivity during the day is likely to be due to the progressive opening of flowers during the night. This elongated period of receptivity potentially contributed to overall seed set, due to the presence of nectar feeding birds, which may affect pollination. The overall reproductive output of the Walkamin population appeared to be slightly greater than others reported elsewhere, though comparison was made difficult by a lack of consistency in the expression of the variables measured.

Conclusion

While the previous success of seed production at Walkamin was repeated and confirmed in detail, and while it is certainly more successful than that reported informally from many sites round the world, the record from three other carefully monitored sites suggests that the same order of production is achieved elsewhere. Whether or not the same order of recovery of seed is also obtained is not recorded, but it is relevant to point out that the success of *Calliandra* seed production at Walkamin has been attributable at least as much to efficient recovery as to production.

It became clear that Walkamin's yields, however good in relative terms, could be considerably increased with further attention to tree spacing, and that overcrowding was a major factor in limiting production. Inflorescence populations and pod set appeared to be critical variables. Andromonoecy was not a serious limitation to productivity.

We believe that the basis of success of success at Walkamin has been, first, the use of a climate suitable for a wide range of legume seed production; second, the application of management practices not greatly different from those used with herbaceous legumes, and particularly the manipulation of the timing of flowering through pruning so that it occurs at a favourable time for both effective seed set and reliable seed ripening; and, third, the use of efficient seed recovery methods replacing the usually inefficient hand-picking. The first two, however, only put in place a dense, vigorous population of inflorescences, and while the circumstances may enhance pollination, they do not on their own allow it to occur at a sufficient frequency to realise a heavy seed crop. This is the task of the pollinators, without which it seems that only limited success would be possible, and it is the flying foxes that seem to be most important in this role.

What lessons are there to learn from this exercise for people faced with failure of much-needed seed production in other parts of the world? Our experience of weak flowering at nearby Kairi and of legume seed production generally, makes us emphasise the choice wherever possible of a suitable climate, particularly with a reliable dry season. We further obviously attach importance to management, with emphasis on tree spacing as well as pruning, etc., and later to alternatives to hand-picking of seed. We would recommend attention to prospective pollinators, particularly to the role of bats. If, as is reported in many parts of south-east Asia, flying foxes are few, thought could be given to nocturnal hand-pollination of inflorescences. Where labour is cheap, it is not inconceivable to visualise imitating the action of flying foxes with pollen-collecting surfaces of wool or fur on the ends of poles. At every stage, we would suggest monitoring, particularly of inflorescence and pod populations, in order to get some analysis of the system, however rudimentary.

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Appendices

Appendix A: File details

All data (Microsoft Excel), report (Microsoft Word), photographs of the Walkamin *Calliandra* population and video footage displaying the feeding behaviour of the tame Spectacled Flying Fox and Queensland Blossom Bat on *Calliandra* inflorescences at Walkamin are available. Material is lodged with John Hopkinson, Walkamin DPI, Queensland, Australia and Alan Pottinger, Oxford Forestry Insititute, Oxford University, UK.

File Details

infl#98.xls: file contains data of inflorescence numbers from whole tree counts collected at two different times during season

fa1298.txt: file contains data of floret numbers and andromonoecy numbers over season. Three collections were made however time 1 is the only fairly complete data set, times 2&3 were troublesome and so are incomplete for different trees.

fa1298.xls (sheet 1): raw data and some summary calculations - results from these calculations are located in sheet 2.

fa1298.xls (sheet 2): summary of floret and andromonoecy counts for a given tree at a given position (1=lower, 2=upper). Each value is the result of summing-up of the particular values in a given inflorescence. Each value therefore represents the total florets or andromonoecious florets for a given inflorescence at a given position within at tree.

seed798.xls: file contains data of pod and seed numbers from whole tree harvests

seed798.xls (sheet 1): summary of the number of seeds or aborted seeds per pod (whole tree counts). The numbers are the result of the first pod harvest (8/7/98) - not all trees had mature pods and only mature or green but hard pods were harvested

seed798.xls (Sheet 2): summary of number of pods used from sub-sample of total pods for a tree (collected 29/8/98) in order to perform seed counts. Table also includes proportion of useful pods (ie developed pods/total pods) for that tree and the proportion of total pods for a given tree that would give useful seed counts (ie. total pods for a tree*prop.useful pods)

seed798.xls (sheet 3): Summary of the total number of pods collected (whole tree counts) for the season (includes two harvests)

seed798.xls (sheet 4): summary of the number of seeds or aborted seeds per pod. Whole tree harvests were performed (29/8/98), a sub-sample was taken and then mature pods from that sub-sample counted. Details of the sub-sample numbers are summarised on sheet 2.

seed798.xls (sheet): raw data

ltep998.xls: Long term exclusion experiment comparing the number of PODS produced from continuous cover by bird/bat proof (Ha!) cages (cbp), continuous cover by insect proof bags (cip) and continuously uncovered inflorescences (cu)

ltep998.xls (sheet 1): raw data

ltep998.xls (sheet 2): summary of long term exclusion experiment - number of PODS produced for each exclusion type

ltes998.xls: long term exclusion experiment - number of seeds/aborted seeds produced for each exclusion type (same as above ie cbp, cip and cu)

ltes998.xls (sheet 1): raw data

ltes998.xls (sheet 2): summary table of number of seeds/aborted seeds produced for each exclusion type

step998.xls: short term exclusion experiment comparing the number of PODS produced from night exclusion (covered during night, exposed during day, n): bat exclusion (nbp), night insect exclusion (nip), day exclusion (covered during day, exposed at night): day bird exclusion (dbp), day insect exclusion (dip), continuously covered with either cages (bird/bat proof, cbp) or bags (insect proof, cip) and continously uncovered (cubp and cuip)

step998.xls (sheet 1): raw data

step998.xls (sheet 2): summary table of number of pods produced for each exclusion type

stes998.xls: short term exclusion experiment comparing number of seeds and aborted seeds produced from different exclusion treatments (treatments as above, nbp, nip, dbp, dip, cbp, cip, cubp, cuip)

stes998.xls (sheet 1): raw data

stes998.xls (sheet 2): summary table of number of seeds and aborted seeds produced for each exclusion type

nbsr698.xls: Nile blue test for stigma receptivity results. Ten times are listed (details of times 1-10 are summarised in table within report). Region stained, staining intensity and percentage of pistils stained (percentage of total viewed).

nbsr698.xls (sheet 1): raw data

nbsr698.xls (sheet 2): summary table of each measure for each time, also includes some graphs of data.