

Karri
(*Eucalyptus diversicolor* F. Muell.)
Phenological Studies in Relation
to Reforestation

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Summary

The supply of seed for regeneration of karri and the periodic yield of honey are governed by many factors, including genetics, habitat and weather. This study describes detailed observations of karri floral cycles over the decade 1956 to 1967, and less detailed observations of crops of karri honey and seeds since 1926. The floral cycles and seed and honey crops are related to the cumulative rainfall.

The study indicates that inflorescence initials appear almost every summer, but that their development into seed crop is uncertain due to frequent losses of flower buds and immature fruits. The interval between appearance of the initials and of the full blossom varies from one and a half to almost three years. The interval between the appearance of bud initials and the ripening of seed is three years for spring into summer blossom, and four years for autumn into winter blossom. More than half of the viable seed is shed in the summer immediately after ripening and the balance is usually shed early in the following summer.

Periodic seed production varies more than that of the blossom, because of high cumulative losses before maturity. Whereas two consecutive crops in one tree may ripen in phase over one winter, one year's crop in different trees may extend the ripening period over two or three years. Intervals without seed can also be long and variable. The presence of seed on trees for a period of one to three years may be followed by three or more years without seed. These fluctuations are quite irregular.

The seed supply is assessed from the number of capsules in the crown, the number of seeds per capsule and the percentage of crown cover. In a good seed year four seed trees per hectare, corresponding

to approximately 15 per cent crown cover, can provide an adequate regeneration seed supply of 200 000 seeds in autumn or 300 000 seeds in spring.

The direct sampling in the crown of the trees, using telescopes and rifles with telescopic sights, is preferable to indirect prediction using sampling trays, in which the seed crop is inferred from progressive shedding of floral components. However, sampling trays are useful for assessing the dispersal of seeds from the seed trees, though this information can only be used to assess whether the seed shed is adequate, or whether natural regeneration needs to be supplemented or replaced by artificial regeneration.

Poor seed set, resulting in only one or two seeds per capsule, is prevalent in karri and there is clearly a need for improvement. Pollination by introduced bees and manipulated crosses doubled the number of seeds per capsule in a limited number of trials.

Honey flows lasting three to five months yielded up to 45 kg per month per hive of 60 000 bees. An apiary site of 800 ha in karri forest may be expected to support 40 hives in light blossom, 120 in moderate blossom and 600 hives in a prime season. Prime honey flows occur only once in four to twelve years.

Seed collection is most economical during these occasional prime crops. Collection can be commenced when the seed ripens nine months after the flowering. Fifteen to twenty trees can produce one tonne of capsules containing 30 to 40 kg of chaff and seed—16 per cent purity. This yields 5 to 6 kg of pure seed at 700 000 seeds per kilogram. Depending upon the techniques and density of regeneration desirable, this seed is sufficient to broadcast-sow 30 to 60 ha or to plant 1 000 to 2 000 ha with seedlings raised in the nursery.

Leaf and Floral Cycles

INTRODUCTION

Initial investigations into the seeding habits of karri (*Eucalyptus diversicolor* F. Muell.) were carried out on mature trees which had been topped and spiked to serve as fire look-out towers. Crown studies were restricted to the three most accessible trees, viz. Gloucester Tree, Diamond Tree and Pemberton Tree (Fig. 1). These trees, 45 to 60 m high, were studied in detail from 1957 to 1967.

At the beginning of the study there was an abundance of fruit in the epicormic crowns of the look-out trees and in the primary crowns of the adjacent undisturbed trees. This confirmed that the leaf nodes of karri shoots, whether of primary or secondary (epicormic) origin, were capable of producing leaf buds, leafy shoots, inflorescence and fruits (Jacobs, 1955). It was concluded, therefore, that the lopping of the look-out trees had no effect on their leaf and floral cycles.

METHOD

On each look-out tree the positions of three or four strong branches were recorded and healthy branchlets were labelled with numbered metal tags. In 1957 the leaves of three or four terminal branchlets on each branch were marked with a revolving punch-plier. Each subsequent summer, further mature leaves were marked with large punch-holes in the odd-numbered years and small punch-holes in the even-numbered years. Each February, May and November the numbers and stages of development of leaf and floral components were recorded. Samples representing the observed stages of fruit and floral development were taken from adjacent, unmarked branchlets for a record. Capsule maturity and seed yield per capsule were checked regularly.

Diagrams of the karri floral cycle and its variations were prepared according to Moggi's (1958) representation of eucalypt floral cycles. A detailed recording of the leaf and floral behaviour has made it possible to interpret the development of seed and ultimately to incorporate this knowledge into regeneration practice.

RESULTS AND DISCUSSION

Branch development

Karri leaf and branch growth follow the pattern described by Jacobs (1955) for eucalypts in general and by Stoate and Wallace (1938) for jarrah (*Eucalyptus marginata* Sm.) in particular.

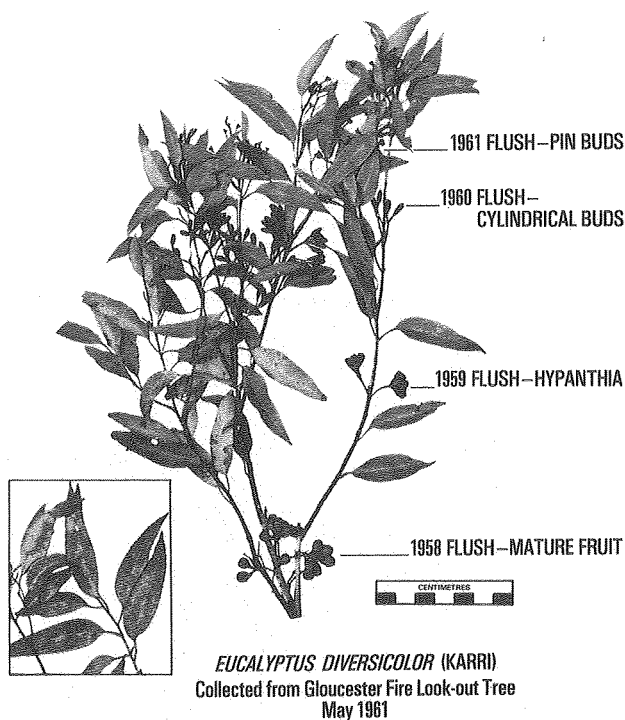


Figure 2

Leaf growth and floral development

1961 flush—pin buds	1959 flush—hypanthia
1960 flush—cylindrical buds	1958 flush—mature fruit

The inset shows basic leaf arrangement.

Incipient flush usually appears on the terminal branchlets of karri crowns early in November. During the growth of a branchlet, leaves unfold from the naked bud of the growing tip and also from the axillary naked buds formed in the axil of each leaf. The leaves of these branchlets unfold from the naked buds in an alternate, sub-opposite pattern; between leaves of a pair the internode is short and between pairs the internode is relatively long (inset, Fig. 2). The leaves and shoots reach their ultimate length by the end of January and the leaves thicken and harden during February. Throughout this period of growth a large number of leaf buds and immature leaves fall off, apparently nipped off by insects.

Branchlets are produced during the simultaneous and ramifying growth of the axillary shoots. The first side shoots, which arise directly from the leading shoot, form branchlets of the first order and are more vigorous than subsequent side shoots of a higher order. The second and higher order branchlets (rarely more than five or six orders from the main stem) are

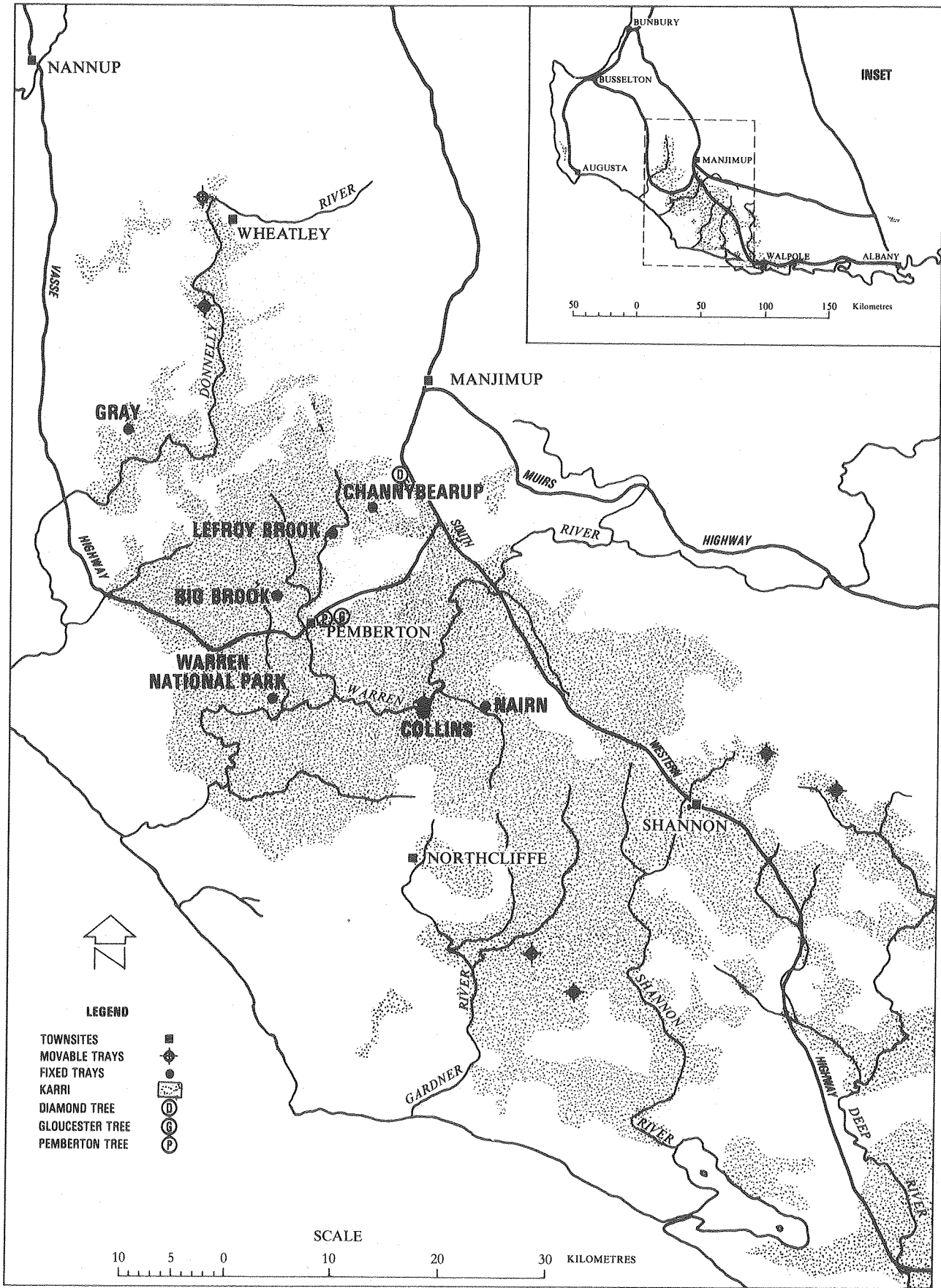


Figure 1

The study area and outlying forests.

less dominant and progressively weaker. Forks are designated the same order when they make two or more competing branchlets of the same size from the same shoot. A leafy branch is a crown unit and consists of the growth of its main shoot and of the short-lived branchlets, which on dying leave a clean length of stem behind the growing unit. A single branchlet, or twig, consists of all the components directly attached to it normally on stalks. When the leaves die, the branchlets die and all the components are shed.

Longevity of leaves

Of the 550 leaves marked for observation in the three look-out trees, 69 per cent (± 6 per cent) lived for one year, 24 per cent for two years, 5 per cent for three years and 2 per cent for four years. However, this pattern will vary depending upon the weather and vigour of the shoot in relation to its position in the tree and to the abundance of its floral development.

The early order branchlets, which continued to maintain vigorous growth, flushed strongly and shed leaves rapidly, whereas the late order branchlets ceased growing and retained leaves for a longer period. Heavily fruiting branchlets ceased growing during the period from floral bud development until seed maturity and retained leaves for the longest period, which coincided with the duration of the floral cycle.

Floral development

Inflorescence initials form in the terminal axils early in summer. Two bracts enclose each inflorescence, which becomes a globose swelling on the apex of the peduncle. The inflorescence lengthens by 1 to 2 cm and bears seven floral bud initials, which can be distinguished when the bracts are cast at the end of March. Plate 1 shows monthly stages of the karri floral cycle.

Floral bud development is slow and rate of growth irregular; growth is almost completely arrested in winter when temperatures drop. At this stage the length of a floral bud and pedicel (pin bud) is 0.5 to 1.0 cm. In spring bud development resumes and by autumn the full length of about 2 cm is attained. At this stage (twelve to fifteen months from inflorescence) the floral buds are called cylindrical buds and have green, conical bud caps. Growth again ceases during the second winter. During the third summer the buds become plump, and are described as clavate. Before flowering these clavate buds turn yellow-green to pale brown, and their opercula (caps) become dome-shaped and reddish-brown.

Blossom

Approximately one week before flowering a white abscission line appears at the junction of the operculum and the hypanthium. Shortly afterwards the operculum falls off, exposing the stamens. The

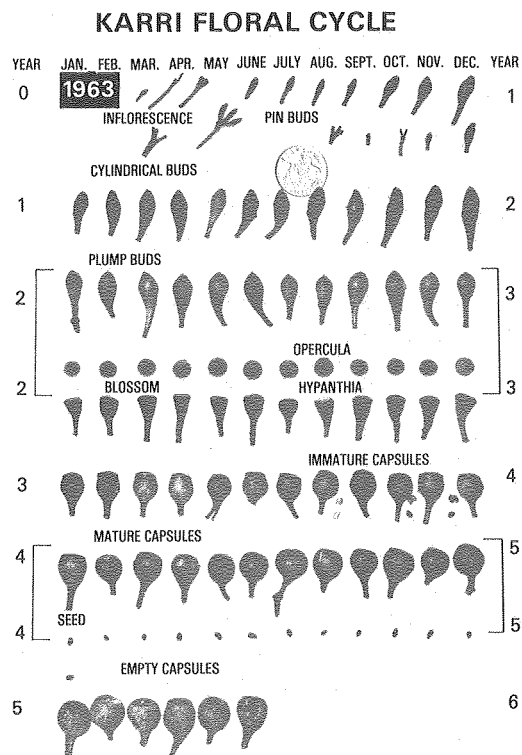


Plate 1

Year and stages in the floral cycle from 1958 to 1963:

- 0-1 Inflorescence and pin buds of 1958
- 1-2 Cylindrical buds of 1959
- 2-3 Clavate (plump) buds of 1960
- Blossom with separation of opercula from hypanthia (immature receptacles)
- Stage 1 fruit—hypanthia of 1960
- 3-4 Stage 2 fruit—immature capsules of 1961
- 4-5 Stage 3 fruit—mature capsules and seed of 1962
- 5-6 Stage 4 fruit—old empty capsules of 1963

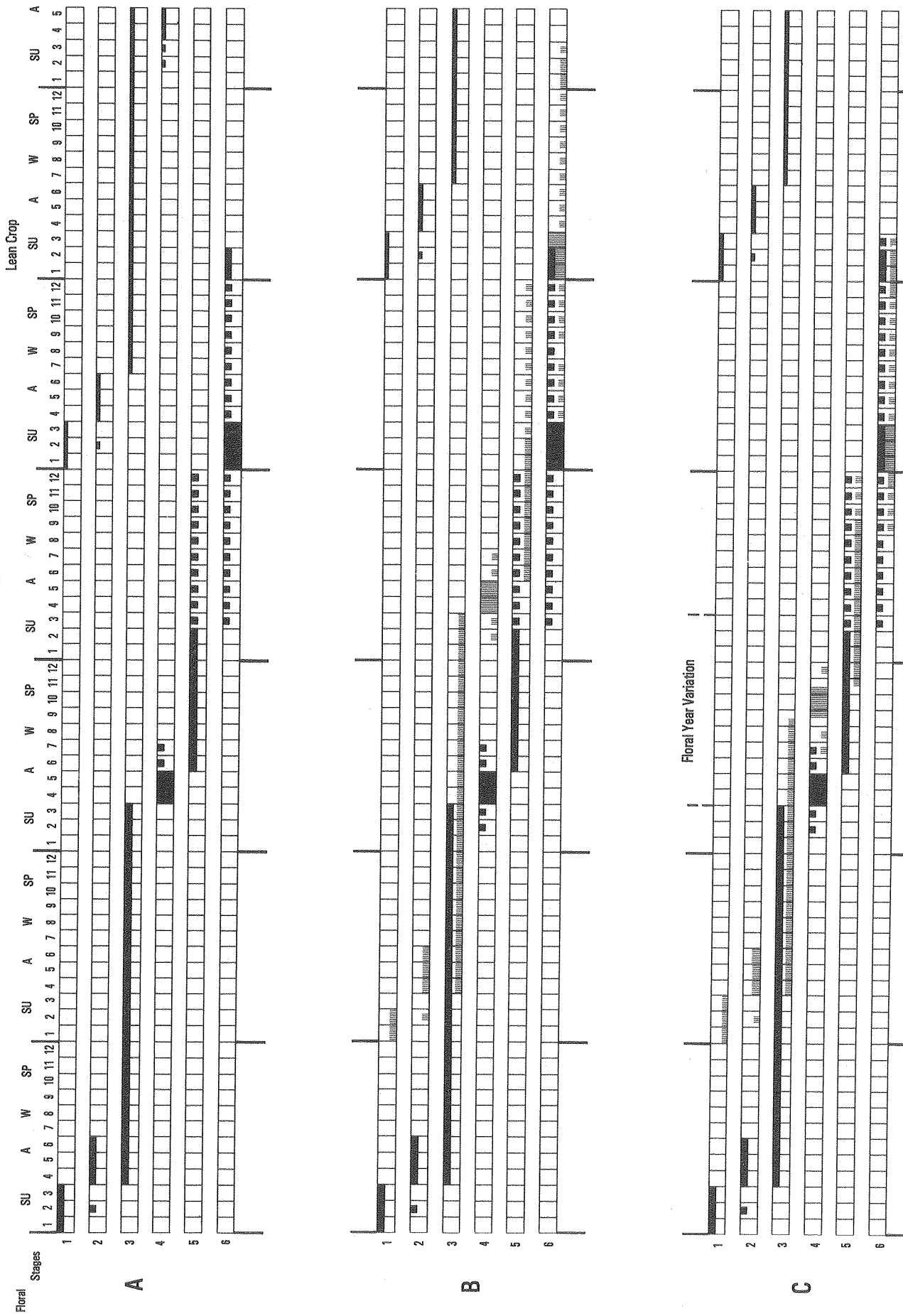
stamens take four or five days to open out and expose the anthers; by this time, the stigma is sticky and ready for fertilisation. After about one week the anthers turn brown and the stamens become depressed, wither and usually fall off. The inside of the hypanthium dries up within one month of shedding the operculum.

A tree may continue to flower for six months, with a peak lasting two to three months. Other flowerbud crops, formed before or after the main one, may or may not flower. The time and season of flowering is difficult to forecast, especially for small crops between abundant crops.

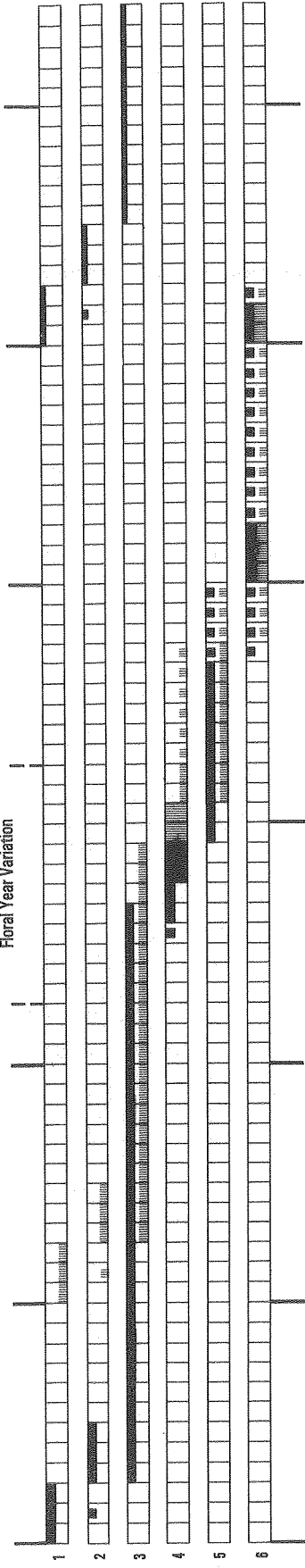
Ripening of capsules

After flowering, the immature cups (hypanthia) continue to develop through one full winter before the seed crop is ripe. The minimum recorded period for the development of blossom into mature seed crop is nine months; this is more common in the summer blossom, the winter blossom normally requiring twelve months to mature.

Figure 3
KARRI FLORAL CYCLE

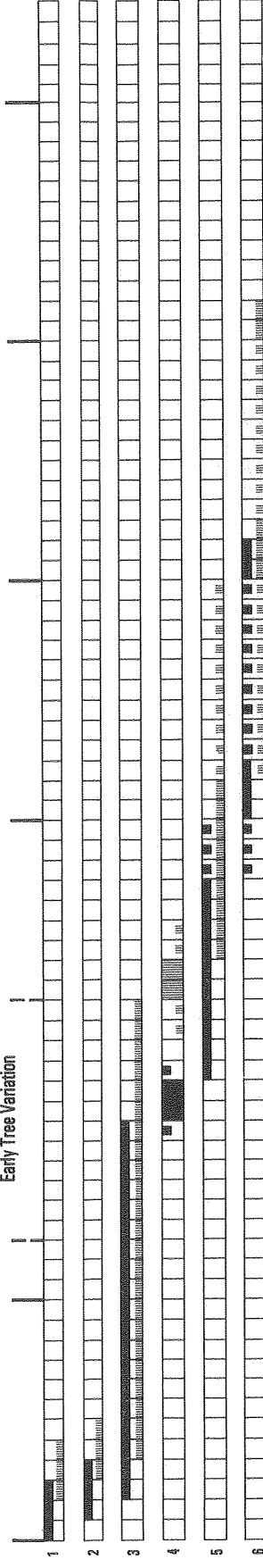


Floral Year Variation



D

Early Tree Variation



E

LEGEND

- First crop
- Second crop
- Lean crop
- Intermittent activity (first crop)
- Intermittent activity (second crop)
- Intermittent activity (lean crop)
- Main blossom or main seeding (first crop)
- Main seedings of first and second crops coincide
- Main blossom or main seeding (second crop)

FLORAL STAGES

- A. Four-year floral cycle from the formation of the inflorescence initials to the main seed dissemination.
- B. Consecutive four-year floral cycles from two successive annual inflorescences.
- C. Winter blossom may occur during autumn-winter in one crop and in winter-spring in another. Autumn blossom in a four-year cycle occurs 2-2.5 to 2-5 years after the start of the inflorescence, and is followed by the second annual inflorescence initials with winter-spring blossom at 1-5 to 1-7.5 years in a three-year floral cycle. Both seed crops ripen in phase in late summer-autumn and mature over winter. Blossom in the three-year cycle is advanced.
- D. Summer blossom may occur simultaneously for two crops (Fig. 4). Blossom in the four-year cycle is delayed. Blossom in the three-year cycle is advanced. The crops ripen in phase during winter-spring.
- E. Three-year and four-year cycles initiated in the same year for trees which have a different rate of bud development (Plate 4).

Formation of the inflorescence initial

1. Forming of the inflorescence—fall of bracts uncovers the pin bud.
2. Development of the pin buds—fall of the calycine operculum of the pin bud.
3. Development of the clavate bud—fall of the corolline operculum.
4. Blossom—fall of the stamens.
5. Ripening of the capsule—start of seed dissemination.
6. Dissemination of the seed—end of seed dissemination.

The seven stages of the floral cycle of the *Eucalyptus* species (Moggi, 1958) and variability of the floral cycles for karri (*E. diversicolor*) are illustrated in Fig. 3. Fluctuations in the quantity of flowering and fruiting are related here to the development pattern of one single or two successive crops.

Seed dissemination related to twig and leaf fall

Seed maturity is associated with three- to four-year-old leaves. Maturity may be assessed by the full shape of the capsules and by the relative position of capsules

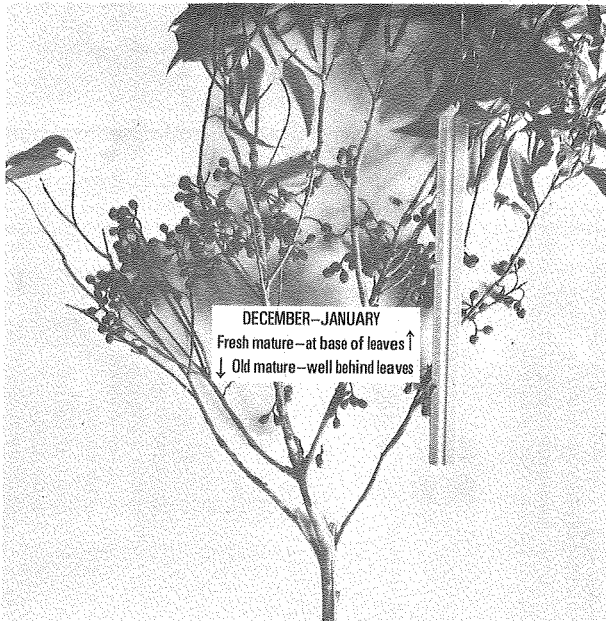


Plate 2

Seed capsules at different stages of maturity:

A. The fruit crop among the leaves is immature; the leading branchlets continue flush growth, while the subordinate side branchlet has discontinued this growth. Also in Fig. 2 the crop becoming exposed at the base of the leaves is mature.

B. Seed dissemination shown at four years from bud initiation, occurs first from capsules on the dead and dying side branchlets (those which did not flush), while the main leafy branches retain foliage growth and their capsules until the next year. Seed dissemination subsequently, at five years from bud initiation, also occurs at this time from the older capsules shown on the main leafy branches.

and leaves: hypanthia and immature capsules are mingled with the leaves, but mature capsules become exposed close to the base of the foliage (Plate 2).

Seed is disseminated when the capsules open, either on the tree, or after the capsules have fallen to the ground. The main dispersal through opening begins when ripe capsules harden and darken. Drying of the capsules and dissemination of seed are related to the fall and flush of the leaves respectively. Timing of dispersal is also influenced by the position of capsules in relation to the main and vigorous side branches, or the weaker side branchlets. On fruiting side branchlets, which die after leaf fall, the capsules turn brown and cast their seed. Capsules attached directly to the main branches and low order side branches, which continue to grow, turn greyish-green or greenish-brown and retain seed for a further twelve months before drying out, disseminating the seed, and then being shed. At this stage, the crop is four to five years old.

Crop variation

Crop variation between trees is controlled by either genetic or environmental factors. Constant differences in the flowering season between trees in the same area indicate genetic control; differences in the quantities of blossom in successive years in genetically related trees indicate environmental influences.

These factors and their interaction either advance or delay the commencement or completion of the main blossom (Table 1). For example, in the virgin stand of Warren National Park, blossom which developed from the 1957 inflorescence initials was delayed into late 1959, and that from the 1959 initials was advanced into early 1961. Bud development and blossom of the two subsidiary crops therefore became so adjusted that their flowering began and ended in phase with the 1958 main crop (Figs. 3, 4). The intensity and frequency of flowering of the individual

Table 1
SEASONAL FLOWERING OF THREE KARRI FIRE LOOK-OUT TREES
(Showing periods of intermittent blossom from spring 1959 to autumn 1961)

Inflorescence initials	Location and flowering times			Phenology (timing of blossom)
	PEMBERTON (early flowering tree)	GLOUCESTER (normal)	DIAMOND (late flowering tree)	
Summer 1957	Aborted 1959	Spring 1959	Spring 1959	Late
Summer 1958	Spring 1959	Autumn 1960	Winter 1960	Normal
Summer 1959	Spring 1960	Autumn 1961	Autumn 1961	Early

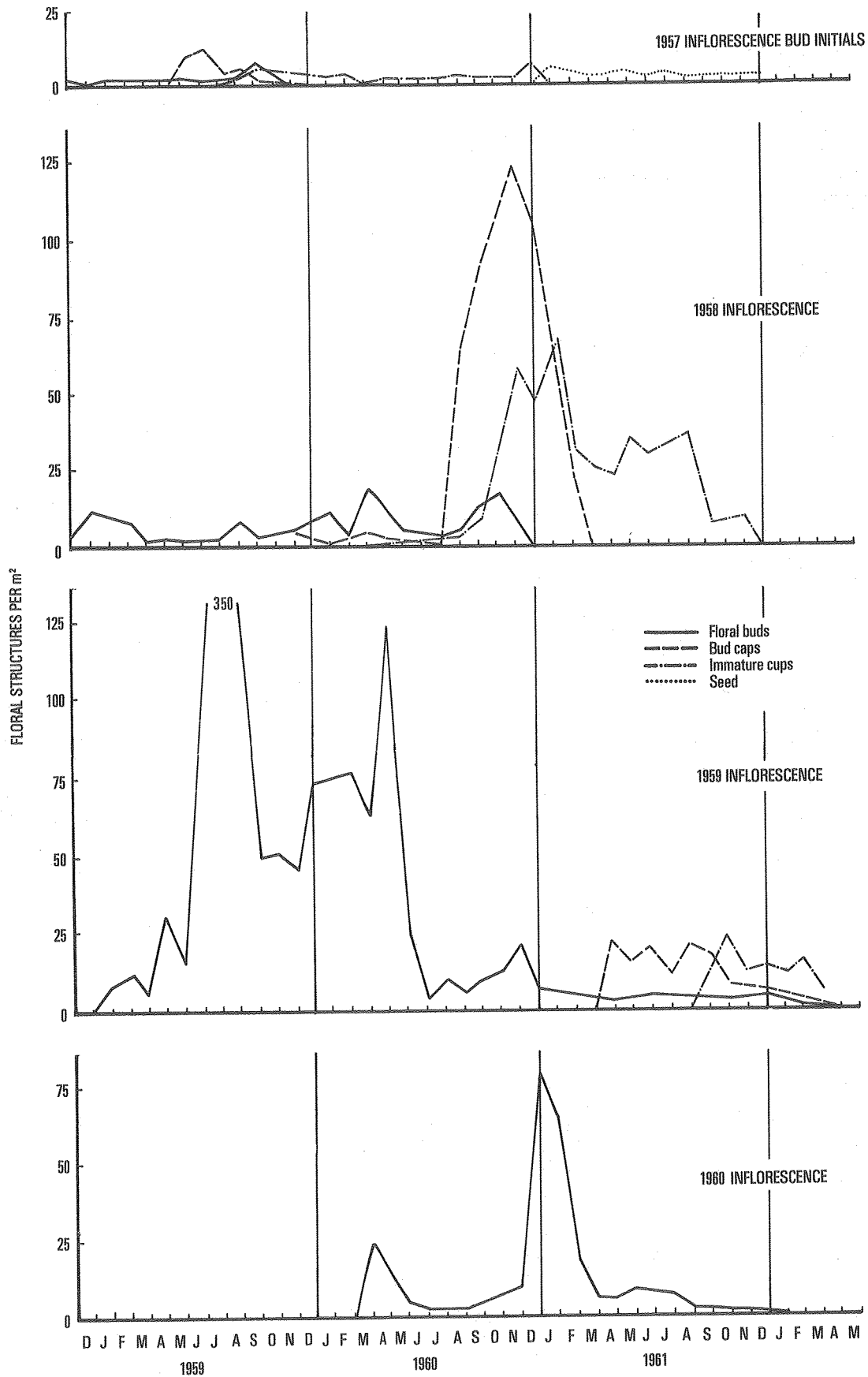


Figure 4

Shed of floral components in a virgin stand at Warren National Park. The bud caps from 1958 and 1959 inflorescence initials could not be distinguished from each other in spring 1960 (the 1961 bud caps originated from the 1959 inflorescence initials).

trees in the stand also fluctuated, so that until the 1960 flowering it was difficult to recognise the main crop.

The observation of floral development of the three look-out trees confirmed a four- to five-year cycle from inflorescence initials to the maturation and dissemination of the seed crop. The comparison of flowering patterns of these trees with those of the other stand indicated that the genetically controlled variations of individual trees harmonised with the environmentally induced expression of a four-year cycle for seed production. The secondary bud crop, which was initiated one year later than the main stand crop, developed more rapidly so that it had only a three- to four-year cycle, compared with the normal four- to five-year cycle.

As observed, karri has either one or two crops of mature seed, seldom more, on a tree at one time. Regular development of two crops with twelve months between them is shown in Plate 1; however, development is rarely as even and regular as this. Regular development of four crops with twelve months between them is shown in Fig. 2. Only the dominant crop retained seed over the unusually long period of four summers before the last of the seed was shed in summer 1964. This abundant crop, observed on Gloucester Tree, developed from the summer-autumn blossom of 1958. The development of fruit in separate crops after summer-autumn blossom is also shown in Fig. 2.

Completely irregular inception of inflorescences may take place in the axils of leaves associated with another bud crop one year older (Plate 3). In this development, rates of growth of separate crops adjust so that they flower in the same season.

Single crops can have different rates of development during the first months (Plate 4). For example, after initiation in summer 1964 advanced bud development in the early-flowering Pemberton Tree, indicating the spring-flowering three-year cycle, contrasted with the autumn-flowering four-year cycle of the Gloucester Tree.

There is always at least one stage of a developing crop, and up to five stages of successive crops may be present, on one branch, in any one year. One crop may be lean, another heavy and another may not develop at all. Seed may be produced in three instead of four or five years. Inflorescence may occur every year, but full development of mature seed may not occur more than once in five years.

As soon as the seed has ripened in one cycle, leaf flush and the development of inflorescence are normally renewed, ready for another cycle. However, the interval between successive floral cycles may vary according to effects of the environment.

CONCLUSION

The floral cycle of karri is complex, with variations between trees and seasons, within crops on one tree, and between stands within a forest. Main blossoming may be interrupted, advanced or delayed depending upon the interaction of genetic and environmental factors. Up to five stages can be found on one tree at any time. Usually one or two crops in every four to seven set good seed and only vestiges of the others remain. Two crops on one tree usually form in consecutive years. Fluctuations in the development of the floral bud and in the season of blossom determine the variable length of the three- or four-year floral cycle.

Plate 3



A. Irregular inception of recent flowerbud initials in leaf axils of two consecutive annual flushes (1958-59). Leaves removed from 1959 specimen.

B. Close-up view of irregular bud initiation in the recent crop (1959) in any position of the previous crop (initiated in summer 1958).

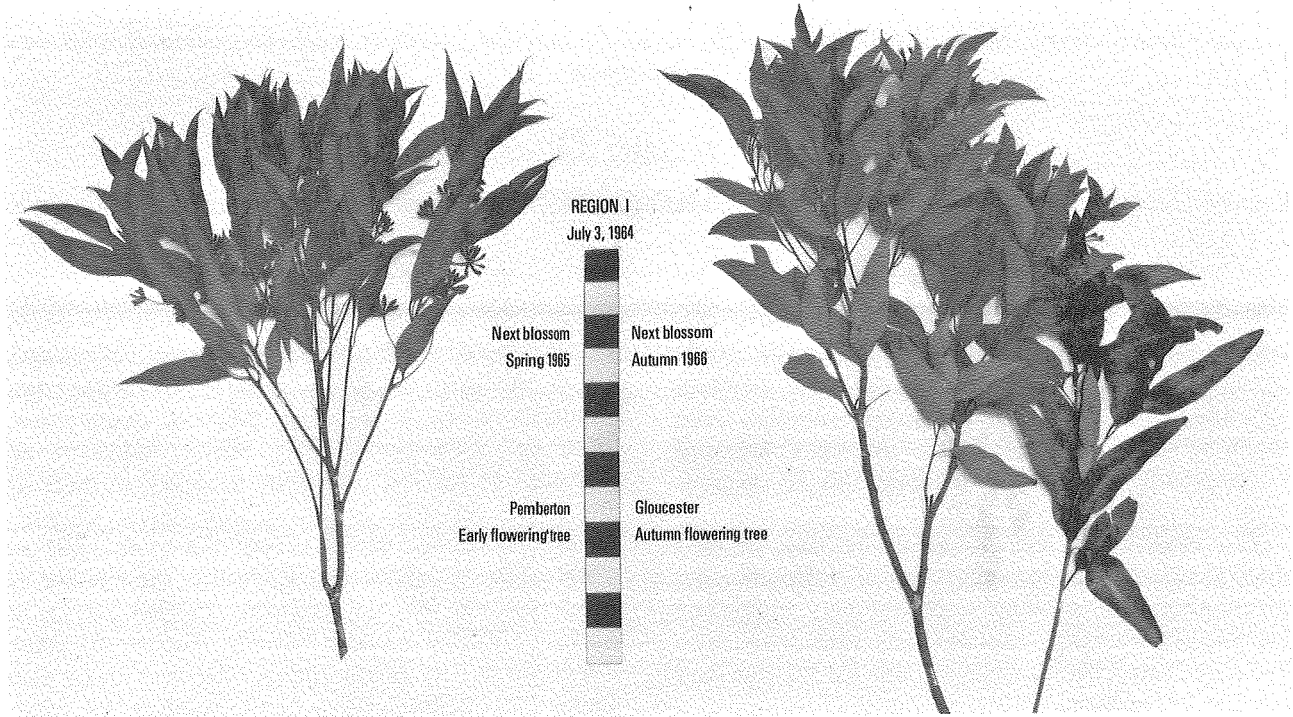


Plate 4

Pin bud development of Pemberton and Gloucester Trees from initiation in summer 1964 showing (Pemberton Tree, left) advanced bud development indicating spring blossom and a three-year cycle, and (Gloucester Tree, right) delayed bud development indicating later autumn blossom and a four-year cycle. (One division of scale equals 2.5 cm.)

Seed Production and Dispersal Studies Using Sampling Trays

INTRODUCTION

Karri floral components and seed, and their dispersal, were sampled using seed trays. This study aimed to record the floral cycle in stands of different ages and structures, to estimate seed production from the blossom using the methods of Ashton (1956) and Cunningham (1960), and to relate this to requirements for natural regeneration.

The condition of the ground at the time of seed dissemination is an important factor affecting seed germination and seedling establishment. Prescribed burning is planned to coincide with seed production and to prepare a fresh ashbed suitable for the ripe seed released by the burn.

After dissemination, seed germination begins in the autumn, usually within six weeks of opening seasonal rains, or more specifically after 40 mm of rain in three consecutive days. In one survey conducted during 1956 and 1957, the tree survival at one year old from seed which germinated and established, ranged from 6.3 per cent on fresh ashbed to 2.7 per cent on clean surface soil. This contrasted with a poor tree survival (0.6 per cent) on unreceptive sites, either compacted or unburnt. The heavy loss (73 per cent) of germinated seedlings shown in Table 2 is attributed to winter fungal attack, animal or insect damage and summer drought in approximately equal proportions (Loneragan, 1961 and 1971).

It was necessary to determine the number of seeds required for adequate regeneration. The density could then be related to the known tree survival percentages of the studies by D. W. R. Stewart (personal communication), D. R. Lejeune (personal communication) and Loneragan (1961), which indicated an average tree survival on freshly prepared ashbeds of 0.6 to 2.8 per cent. The acceptable density of well-spaced established seedlings was taken as 1250 to 2500 per ha. To achieve these densities the number of seeds required was approximately 300000 per ha in spring or 200000 per ha in autumn. This would allow for losses due to disease, pests, adverse weather and competition between plants. Seed numbers for other desirable stockings may be calculated allowing 150 seeds (120 to 180) for each seedling.

METHOD

Sampling

Floral components shed into trays under the karri forest were collected each month from 1956 to 1967. The components were: inflorescence initials, pin buds, cylindrical buds, clavate buds, opercula (from flowers), hypanthia and truncate-ovoid receptacles (immature fruit), brown capsules (recently fallen mature fruit), grey capsules (old mature fruit) and seed. The components were separated and counted,

Table 2
KARRI GERMINATION AND SURVIVAL IN NATURAL REGENERATION

<i>Northcliffe, 1956-57</i>			<i>Seedbed†</i>			
			<i>Fresh ashbed</i>	<i>Clean surface soil</i>	<i>Poor bed</i>	<i>Total</i>
Summer 1957 seeding and winter 1957 germination	Seed bed area sampled	(m ²)	8.8	24.1	14.0	46.9
	Number of seeds	(n)	139	471	205	815
	Germinants counted	(n)	44	34	5	83
	Germination percentage	(%) (a)*	32.6	7.2	2.4	10.2
Summer 1956 seeding and winter 1957 establishment	Seed bed area sampled	(m ²)	10.4	6.9	12.8	30.1
	Number of seeds	(n)	2665	2223	4214	9102
	One-year-old plants counted	(n)	169	60	27	256
	Plant percentage	(%) (b)*	6.3	2.7	0.6	2.8
*Survival rate (b/a) × 100		(%)	19	38	25	27

† Transects 305 mm wide.

and a representative sample was glued to a record sheet. Trays were either in fixed positions in central western stands, or shifted intermittently after selective logging in stands in peripheral regions (Fig. 1).

Fixed trays. Each tray was constructed from woven wire tacked to a wooden frame $610 \times 610 \times 75$ mm and set on 300 mm high stumps topped with antcaps. Ten trays in each stand were arranged in two rows of five trays, with rows 40 m apart, and trays 20 m apart within rows. The grid and its 20 m surround for measuring crown projection occupied 0.96 ha.

The canopy cover (expressed as a proportion of ground area = 1.0) over the sampling area was determined by two methods:

- (1) vertical projection expressed as a percentage of the total ground area;
- (2) convex spherical densiometer (Lemmon, 1957).

The latter method, which takes in some depth of crown, provided a consistent over-estimate of about 10 per cent compared with that obtained from vertical projection. All canopy densities were therefore recorded by vertical projection.

The fixed trays were set up in six plots in the main Warren River Valley system. One plot was in virgin forest of over-mature trees with 0.63 canopy at Warren National Park and had been burnt by wildfire in the summer of 1950. Two plots were in cut-over stands of irregular crown cover: one in Nairn Forest Block had been cut over in 1954, leaving 0.21 canopy, and was burnt for regeneration in 1956; the other, in Collins Forest Block, had been cut over in 1959, leaving 0.0 to 0.06 canopy, and had been patch-burnt for regeneration twice in 1963. A fourth plot in Channybearup Forest Block had been cut over in 1954, leaving an irregular 0.23 canopy; because it was adjacent to the railway line, this stand had been patch-burnt in 1954, 1955, 1959 and 1964. Two plots in second-growth stands were included: one stand in Lefroy Brook Forest was ninety years old, with 0.87 canopy, and had been given mild prescribed burns in 1951 and 1962; the other, in Big Brook Forest, was thirty years old, with 0.85 canopy, and has been fully protected from fire since its establishment. In Big Brook Forest the trays were distributed randomly instead of on a grid.

Movable trays. In 1960, after timber stands had been selectively logged in the river valleys of peripheral karri forest in Wheatley, Northcliffe and Shannon Districts, trays were placed in them. These trays, moulded from woven wire, measured $635 \times 635 \times 100$ mm. At the time of flowering in each landing, six or eight trays were set out under the fringes of several seed trees. Each month the trays were cleared of contents and moved 60 to 100 m to new locations within the landings. The estimated range of the sampling area was 0.01 to 0.05 per cent of the log landings. The number of trays used for the seedfall samplings in

areas selected for regeneration in 1964 was doubled: the trays were placed in pairs with one in openings between trees and the other under the fringe of the nearest seed tree.

The number of seed capsules per unit area may be calculated from the floral balance. The number of capsules remaining on the trees at any time after flowering is equal to the number of bud caps cast during flowering minus the number of fallen flowers and immature capsules cast after flowering (Ashton, 1956). The total seed supply is then equal to the product of capsules per unit area and number of seeds per capsule.

RESULTS

Seed production was estimated from the numbers of opercula and seeds shed during April and March in the years 1956 and 1957 (Table 3, Fig. 5). The results are shown for two individual sampling programmes, one in ninety-year-old regrowth forest (Lefroy Brook) where fixed trays were used and the other in partly logged old growth forest (Shannon River) where movable trays were used. Results for all samplings were combined to assess the relative abundance and pattern of flowering and seeding in the karri forest as a whole.

Table 3
OPERCULA AND SEED PRODUCTION

Floral years	Opercula (thousands per ha)	Seeds (thousands per ha)	Number of trays
1956 January-March	—	815	50
1956-57 April-March	—	613	50
1957-58 April-March	42	62	40
1958-59 April-March	12	—	40
1959-60 April-March	203	32	50
1960-61 April-March	2365	2	50
1961-62 April-March	1767	47	50
1962-63 April-March	1764	146	60
1963-64 April-March	57	442	60
1964-65 April-March	40	193	50
1965-66 April-March	7930	143	70
1966-67 April-March	6920	788	60

The 90 per cent of immature fruit which was shed confirmed observations of extremely poor seed set. For example, in thirteen years (1953 to 1965) the number of opercula exceeded the number of seeds by nearly eight times. For different localities the ratio of opercula to seeds ranged from four to one to thirty to one (Table 4).

Tray sampling in forty-four widely dispersed cut-over stands between April 1960 and March 1961 provided the following floral balance (numbers per hectare):

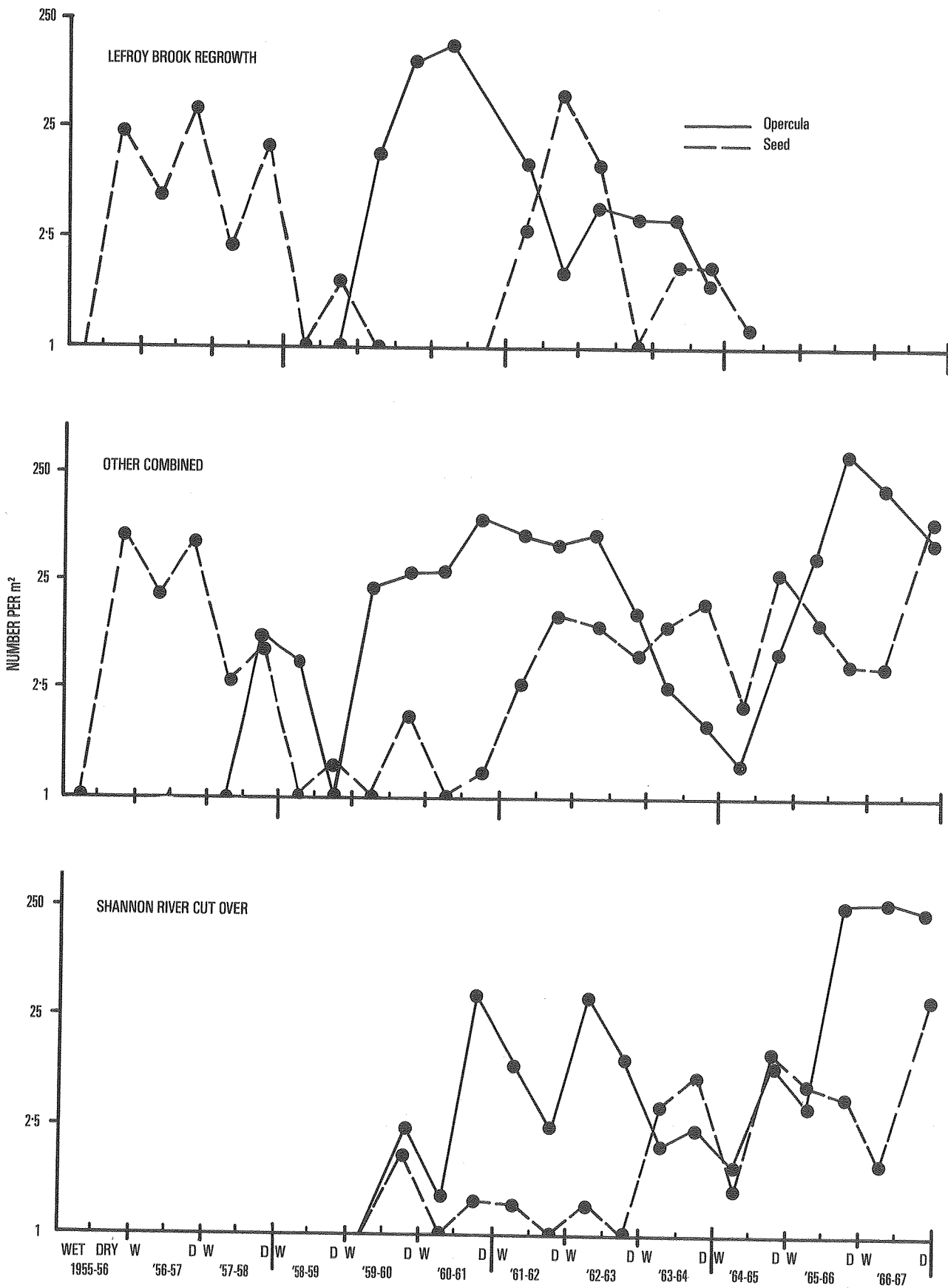


Figure 5

Opercula and seed fall; the centre graph is a combination of measurements from seven stands, excluding the Lefroy Brook and Shannon River stands (logarithmic scale was used for easier comparison).

Opercula	2965000
Less Hypanthia shed during flowering	<u>915000</u>
Equals Immature capsules retained on trees at the completion of flowering	<u><u>2050000</u></u>

Sampling in a virgin stand at the same time, but extended to observe maturing of the seed crop, provided the following figures (numbers per hectare):

Opercula	5 500000
Less Hypanthia and immature capsules shed during flowering and maturing	<u>5000000</u>
Equals Capsules approaching maturity	<u><u>500000</u></u>

However, even this markedly reduced crop of near-mature capsules is much larger than the ripe crop, indicating that the progressive loss of floral components (Fig. 6) continues up to seed shed. Therefore, any early estimates of seed crop should be viewed conservatively.

On average, adequate seed production occurs about one year in three (twenty times in sixty-seven observations). A good seed production in two successive years tends to be followed by three or four years without seed. A very heavy flowering in 1953-54 resulted in a bumper seed crop of two million seeds per hectare in 1956.

Between mid-1958 and mid-1965 flowering occurred on many trees in some stands and on few trees in others, in any one year. Seed was rare in the stands under observation between 1958 and 1961 inclusive. However, a strong flowering of most trees and stands extended from 1960 to 1963. The peak of flowering

varied from stand to stand, and was apparently independent of weather, occurring in both wet and dry periods of 1961 and 1962. The interval between flowering and seed shed varied: winter blossom was followed by seed in one and a half and two and a half years; summer blossom was followed by seed in one and two years. Most of the seed was shed in the first summer after maturation. Half a million seeds per hectare were produced during 1962 to 1964. In 1962, 50 per cent of the stands in the central karri region and none in the peripheral regions produced seed; in 1963, 70 per cent of all stands produced seed; in 1964 seed production was high in the peripheral regions but low in the central region.

Particularly heavy flowerings of seven million flowers per hectare occurred in 1965-66 and 1966-67. These crops were three times that of any other crop observed (1958 to 1967), and both years exceeded the total blossom produced from 1957 to 1964 (Table 3).

The main flowering occurred between September 1964 and August 1966, with a peak blossom and honey flow occurring between December 1965 and May 1966. A few trees and stands continued to flower for a further twelve months. The resulting seed production in 1967 was correspondingly high. There was ample seed supply for natural regeneration in the abundant seed years 1956 and 1967.

Effect of stand density on seed production

Seed production within individual stands varied considerably. This variation was not related to stand density above 0.2 canopy cover. For instance, two cut-over stands of 0.2 to 0.3 canopy produced the

Table 4
SEED PRODUCTION AND BLOSSOM

Location	Stands	Canopy	Date of burning	Opercula (thousands per ha)	Seed (thousands per ha)	Opercula per seed	
Central Karri Forest	<i>First crop</i>						
	(i) Virgin stand, Warren National Park	0.63	27.3.57	NR	2523	NR	
	(ii) Regrowth, Lefroy Brook	0.87	Unburnt	NR	1087	NR	
	Cut-over 1954	Nairn	0.21	30.12.55	NR	3613	NR
		Channybearup	0.23	1.4.57	NR	605	NR
	<i>Second crop</i>						
(i) Virgin stand, Warren National Park	0.63	Unburnt	7660	420	18.2		
(ii) Regrowth, Lefroy Brook	0.87	6.4.62	3514	820	4.3		
Cut-over 1954, Channybearup	0.23	21.11.68	4695	717	6.6		
Peripheral Karri Forest	Cut-over recently	Wheatley	0.2	1.4.63	6509	741	8.9
		Northcliffe	0.2	NR	4366	143	30.5
		Shannon	0.3	18.1.64	8985	1384	6.5

NR = not recorded

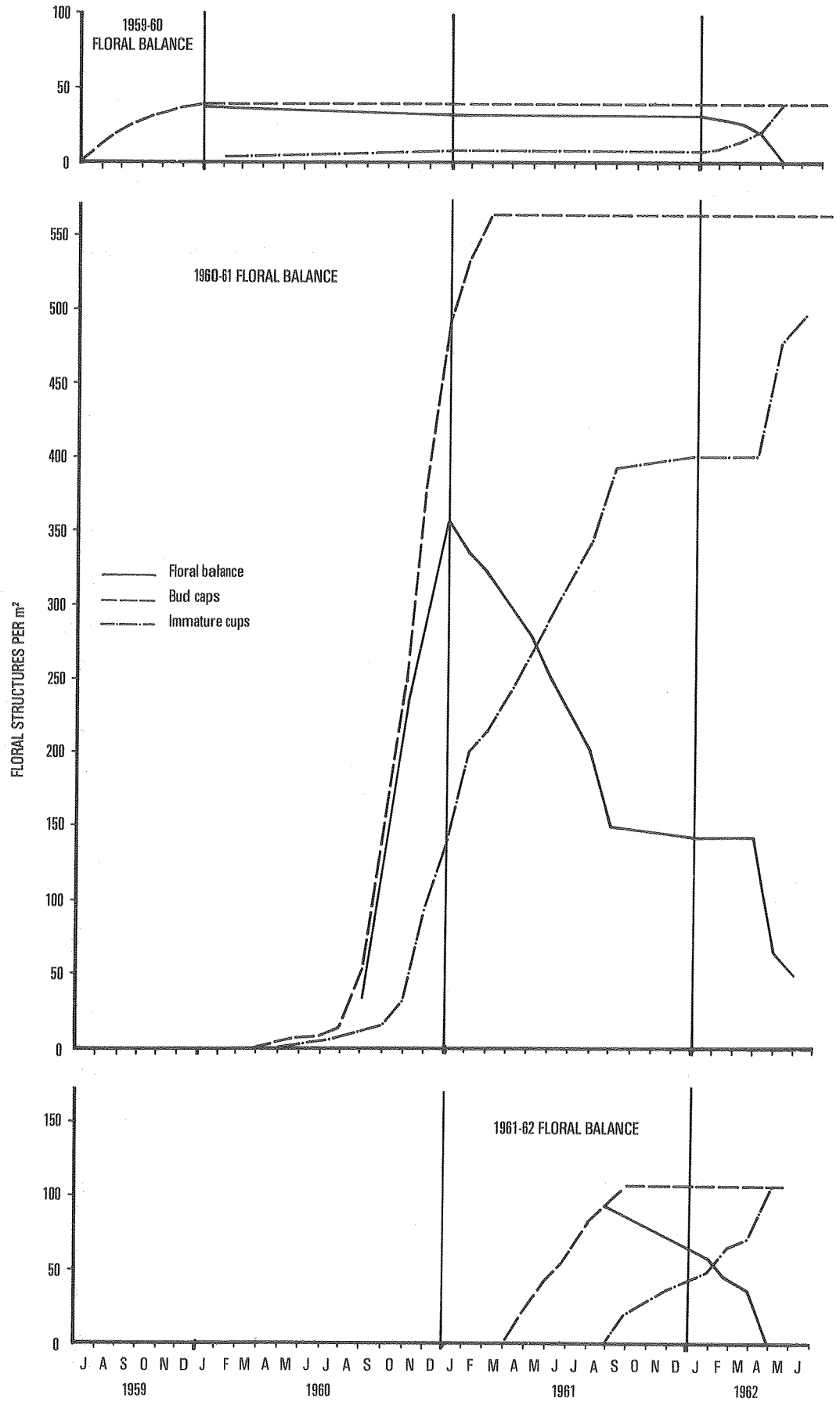


Figure 6
 Variations of floral balance in three crops in a virgin stand at Warren National Park.

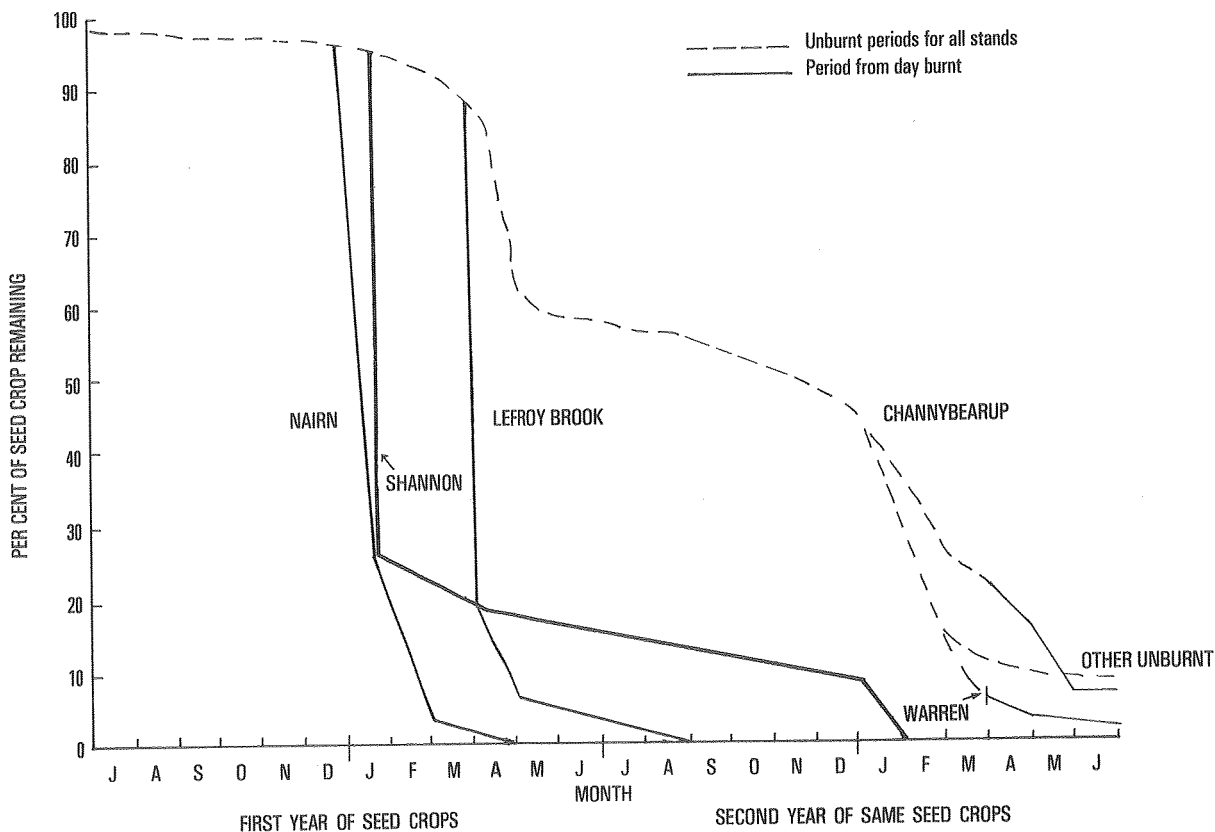


Figure 7

Seed shed as a percentage of total seed crops before and after burning.

same amount of seed per unit area as two well-stocked stands of 0.6 to 0.9 canopy. In contrast, one cut-over stand and a virgin stand produced one and a half times the amount of seed produced by a dense ninety-year-old second-growth stand.

Influence of fire on seed release

In the two well-stocked unburnt stands about 33 per cent of the seed crop was shed in the first summer after seed maturation (Table 4, Fig. 7). Most of the remaining seed was shed in the first autumn and second summer of the crop.

In stands subject to regeneration burns, which scorched more than 50 per cent of the crown of the seed trees, the proportion of the total seed crop shed during the first week after the burn appeared to be influenced by the intensity and season of the fire. A spring burn in the Nairn Forest Block area resulted in 25 per cent of the seed being shed during the first week, 75 per cent during the first month and 97 per cent during the first two months. In contrast, an autumn burn in the Lefroy Brook area resulted in the shedding of 75 per cent of the seed crop during the first week. A mild fuel reduction burn in Warren National Park, which scorched less than 20 per cent of the tree height, did not affect seed shed at all. Generally, the greater the fire intensity, the more rapid the seed shed.

Seed dispersal

During the 1963-64 seed shed, seed dispersal was studied using sampling trays. The seed scattered widely and haphazardly following regeneration burns (Fig. 8). It should be noted that during the seed shed wind varied both in velocity and direction. Despite the relatively large numbers of observations it is not possible to form firm conclusions on dispersal patterns.

CONCLUSION

For successful regeneration of karri stands accurate prediction of the timing and quantity of seed production is essential. Difficulties exist in quantitative assessment of seed production using sampling trays. One of the greatest difficulties is the wide dispersion of seed trees (50 m spacing) in areas prepared for regeneration. To overcome the variation in samples caused by distance of trays from seed trees, a prohibitively large number of seed trays would be required for measuring the floral balance; heavy components (immature fruit) fall closer to the seed trees than light components (seed and opercula). In dense stands this limitation is less significant. Sampling of earlier stages of seed production (buds, opercula, immature capsules) does not aid the advance estimate of seed crop, due to poor seed set and a high proportion of flower and immature capsule losses.

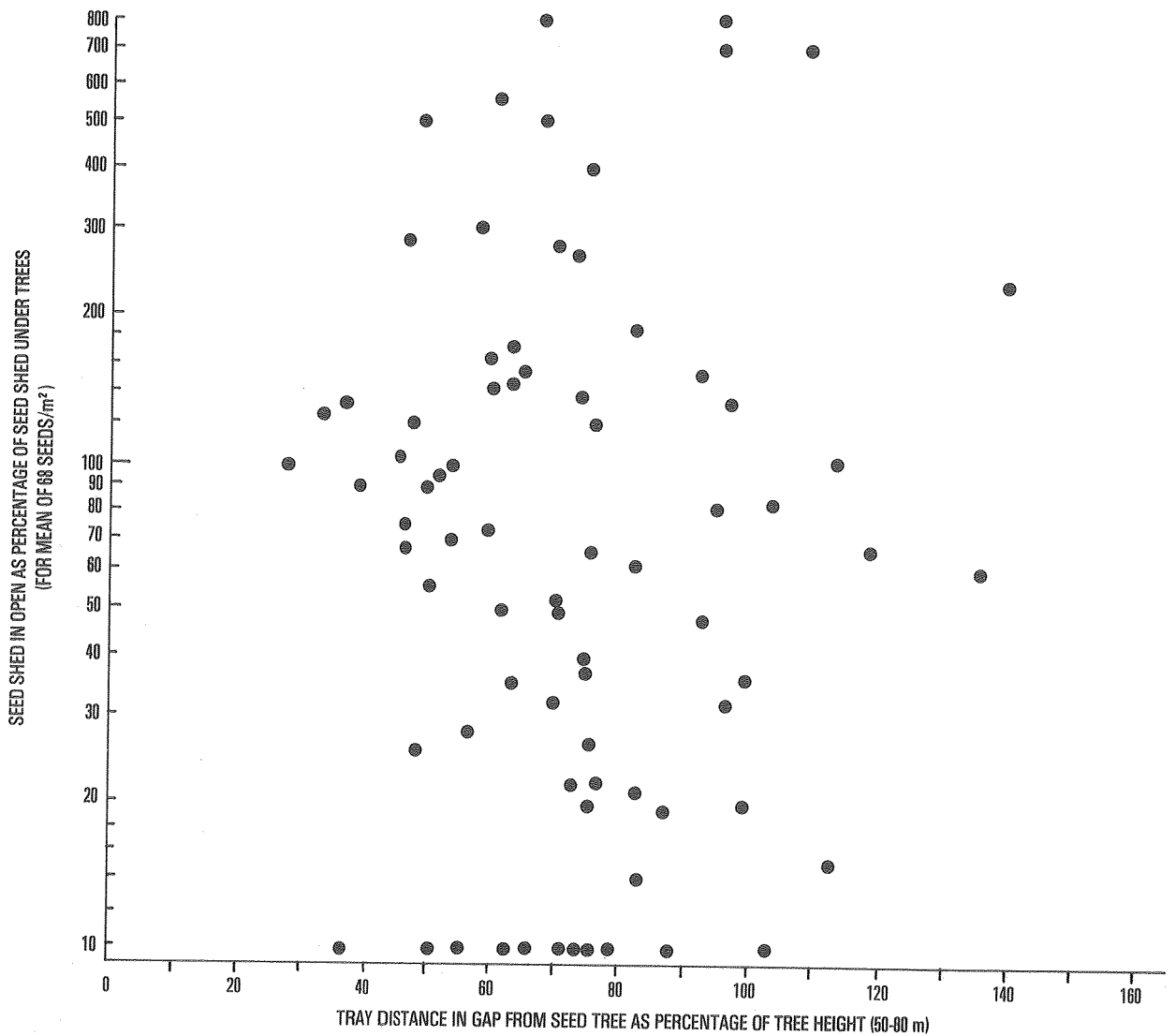


Figure 8

Seed dispersal (in gaps and under seed trees).

Nevertheless, the studies described here have provided a good indication of the flowering and seeding patterns of karri. They indicate that abundant crops occur only infrequently, at intervals of four to twelve years, and must therefore be fully exploited before the seed disperses. Of a full crop available in the spring only 50 to 66 per cent is still available in the

following autumn, and only 33 to 50 per cent is still available in the second spring.

The use of sampling trays to predict seed crops is laborious and time consuming. It is better suited to monitoring the dispersal of the seed crops than to their prediction. Consequently, less tedious and more direct methods of sampling and prediction are needed.

Branch Sampling and Seed Testing

INTRODUCTION

The variation of floral cycles and seed production in karri have been examined (Chapter I), and the use of sampling trays to monitor and predict seed production has been discussed (Chapter II). The use of sampling trays was least efficient in cut-over stands. It is in this situation that accurate and reliable prediction is most necessary so that slash disposal burning for seed bed preparation can be synchronised with seed dissemination from widely spaced residual seed trees. This chapter describes the development of a more direct and reliable method of assessing karri seed production.

METHOD

Collection of capsule samples

Seed production was measured in individual sample trees. Regression analysis was carried out to determine whether seed production was related to tree size. Standard parameters of tree measurements used were: total height, stem diameter, crown diameter, depth and density of leaves (survey density scale of Forestry and Timber Bureau, 1964).

An example of the measurements recorded is shown in Appendix 1. To assess the effect of soil type and tree size on seed production, similar-sized sample trees from two different sites were compared.

The following methods were used to measure seed production on individual trees.

- (1) The capsules on a unit area of the crown of a standing tree were viewed through a telescope and counted (Appendix II).
- (2) An average fruiting branch was shot down from a standing tree, using a rifle equipped with telescopic sight, and the capsules on it were counted. Three average twigs (an average twig was defined as the length of one season's growth) were used as a sub-sample of the whole branch. A relationship between the branch sample and the twig sub-samples was established by counting the number of twigs and the number of capsules within one season's growth of the branches.
- (3) The capsules in the crown of a felled tree were counted, again using twigs as a sub-sample, and then a relationship between the twig sub-sample, the branch samples and the crown as a whole was established.

Finally, the three sampling methods were related to each other. Capsule counting was followed by an assessment of seed yield.

The condition of the seed in the capsule was inspected by cutting a sample open with secateurs.

Yield per capsule and seed testing

It was necessary to establish optimum conditions for seed extraction. Laboratory testing indicated that the germination capacity of seeds was reduced by:

- (1) 5 per cent after drying for one to three days at 45°C;
- (2) 8 per cent after drying for one day at 50°C;
- (3) 50 per cent after drying for three days at 50°C.

Six days at 40°C ($\pm 5^\circ\text{C}$) was adopted as the standard drying schedule for extraction of viable seeds. The capsules were then dried for a further three days at 105°C to determine their oven-dry weight, and to complete the extraction of seed.

The incompleteness of extraction (x) was calculated by the formula:

$$x = \frac{\text{Number of additional seeds extracted at } 105^\circ\text{C}}{\text{Total number of seeds extracted at } 40^\circ\text{C and } 105^\circ\text{C}} \times 100$$

Alternatively, this could be expressed as completeness of the standard extraction (y):

$$y = \frac{\text{Number of seeds extracted at } 40^\circ\text{C}}{\text{Total number of seeds extracted at } 40^\circ\text{C and } 105^\circ\text{C}} \times 100$$

The viability of seed was measured either by the standard laboratory germination method or by a chemical test. In both cases two replicates of fifty seeds each were used for testing.

For the chemical method one-half of a bisected seed was soaked in tetrazolium chloride solution (2.5 g per litre of distilled water) at 20°C for sixteen hours. The viability of the seed was measured by the intensity of staining of the embryo, which reflects its respiration rate (Colbry *et al.*, 1961). The staining varied from bright red for strongly viable seed, to pink for weakly viable, to complete lack of staining for dead seeds. This rapid chemical method, which could be completed in one to two days, gave results comparable to the standard germination technique which requires three to four weeks to complete (Fig. 9).

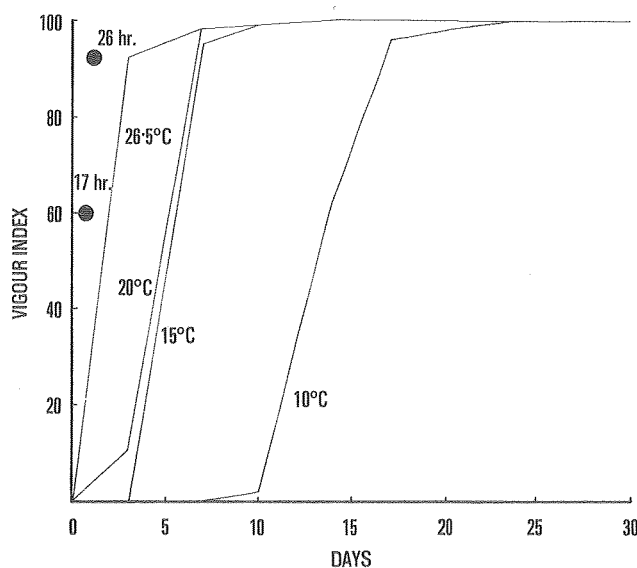


Figure 9

Vigour index of karri seed germinated at four different temperatures. The plots are for the results of chemical tests after 17 and 26 hours.

The following data were recorded during germination testing (Appendices IV and VI):

- (1) Weight per 1000 viable seeds;
- (2) Germination capacity (28 days) and energy (4 days), and the calculated vigour index (energy/capacity);
- (3) Seed purity per cent =
$$\frac{\text{weight of whole pure seed}}{\text{weight of seed plus chaff}} \times 100$$
- (4) Seed size = number per unit weight of sound seed;
- (5) Sound seed number per weight =
$$\frac{\text{number of sound seed}}{\text{weight (including impurities)}}$$

The number of sound seed is determined by a standard squash test: sound seed is indicated by a spot of oil when the endosperm is squashed on white paper. This is useful for setting sample size before testing viability (Appendix III).

RESULTS

Sampling and calibration of parameters

Because of the large variation in capsule moisture content, it is difficult to assess the number of capsules from their weight. Therefore, the weight of, and number of seeds produced by, 1000 capsules (both fresh and oven-dried) was first established. The ratio of weight and number of seeds to weight and number of capsules for this sample was then used to estimate the number of capsules which contributed to the total yield of seed per crown (Appendix I).

The average number of seeds per capsule was 1.2 for the three-twig sub-sample, 1.4 for the branch

sample and 1.7 for the 0.5 kg (about 1000 capsules) sample. The reliability of these sample estimates was not tested.

The branch samples appear to be most reliable, because all sizes of capsules were sampled equally. The three-twig sub-samples consisted predominantly of smaller capsules, whereas the sampler's bias towards selecting larger capsules was evident in the 0.5 kg samples.

The wide range of measurements obtained in the sampling programme made it possible, for the first time, to obtain a number of useful statistics about seed production of karri. The average number of capsules seen in a telescope field in a tree 60 m high was 55.7 per 0.1 m². This corresponded closely to the average number of capsules per three-twig sub-sample, which was 56.3. A tree 60 m high with a bole diameter of 1 m and a crown 20 m wide by 17 m deep and 60 per cent density averaged 393 capsules and 21 twigs per square metre of crown area. These statistics indicate that the number of capsules per square metre of crown area is approximately seven times the number seen in 0.1 m² of the crown in a telescope field, or seven times the number counted on a three-twig sub-sample (Table 5 and Appendix II).

The available data also provided a scale of relative abundance of floral components per twig (Table 5) and a scale of seed supply in relation to the crown cover of seed trees (Fig. 10). This will provide adequate stocking (Chapter II).

Table 5
NUMBER OF FLORAL COMPONENTS PER TWIG
AND PER m²

Scale	Per twig	Per m ² *
Extremely abundant	≥ 39	≥ 800
Very abundant	20-38	400-800
Abundant	10-19	200-400
Frequent	5-9	100-200
Occasional	3-4	50-100
Local	2	25-50
Rare	1	12-24
Very rare	< 1	< 12

* 21 twigs per m² as calibrated with telescope in January 1967 seed crop (see Appendix II and text).

Gross and effective yield

After winter seed maturation the number of viable seeds per capsule increased: in December 1962 the number of viable seeds per capsule was 0.3, but in December 1963 this had increased to 2.0 seeds per capsule. Seed strength also increased with seed maturity: chemical tests indicated that the proportion of strong seed increased from 60 per cent before winter to 80 per cent after winter.

About 17 per cent of the material extracted from the capsules in seventy tests in 1963-64 was pure seed; the remainder was chaff. There were 700 000 pure seeds per kilogram. The average collection yielded 117 000 seeds per kilogram of seed and chaff (16 per cent purity). This corresponded to an average of 1.45 seeds per capsule. Purity increased with increasing seed numbers per capsule but rarely exceeded 30 per cent. Fig. 11 (a) indicates how approximate estimates of seed purity can be obtained by multiplying the number of seeds per capsule by a factor of ten.

The seed weighed between 440 and 990 seeds per gram at 0.95 per cent level of probability. To assess the viability of small seed a sample of 20.85 per cent purity with an average number of 1 150 seeds per gram was sorted into three lots averaging 816 seeds per gram, 1 150 seeds per gram and 2 200 seeds per gram. Chemical tests of the smallest seed showed that it was 87 per cent viable with 75 per cent strong seed. However, it proved inferior in the nursery, probably because of inadequate development of the endosperm and cotyledons.

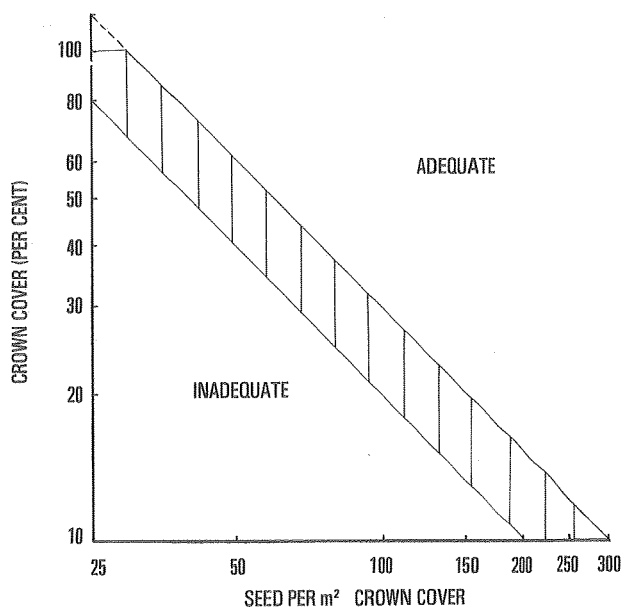


Figure 10

Seed supply limits for natural regeneration at 60 per cent leaf density of crowns. The hatched area is adequate in autumn but inadequate in spring (Chapter II).

Seed yield and capsule weight

To assess these relationships samples of 0.5 kg were used. The relationship between seed yield per capsule weight and seed per capsule is shown in Fig. 11 (b) which shows that the seed yield increases linearly for constant capsule weight as seed per capsule increases. This is an important factor to consider if costs of seed collection are to be reduced. Should the number of seeds per capsule fall below one, it is questionable whether collection is practical.

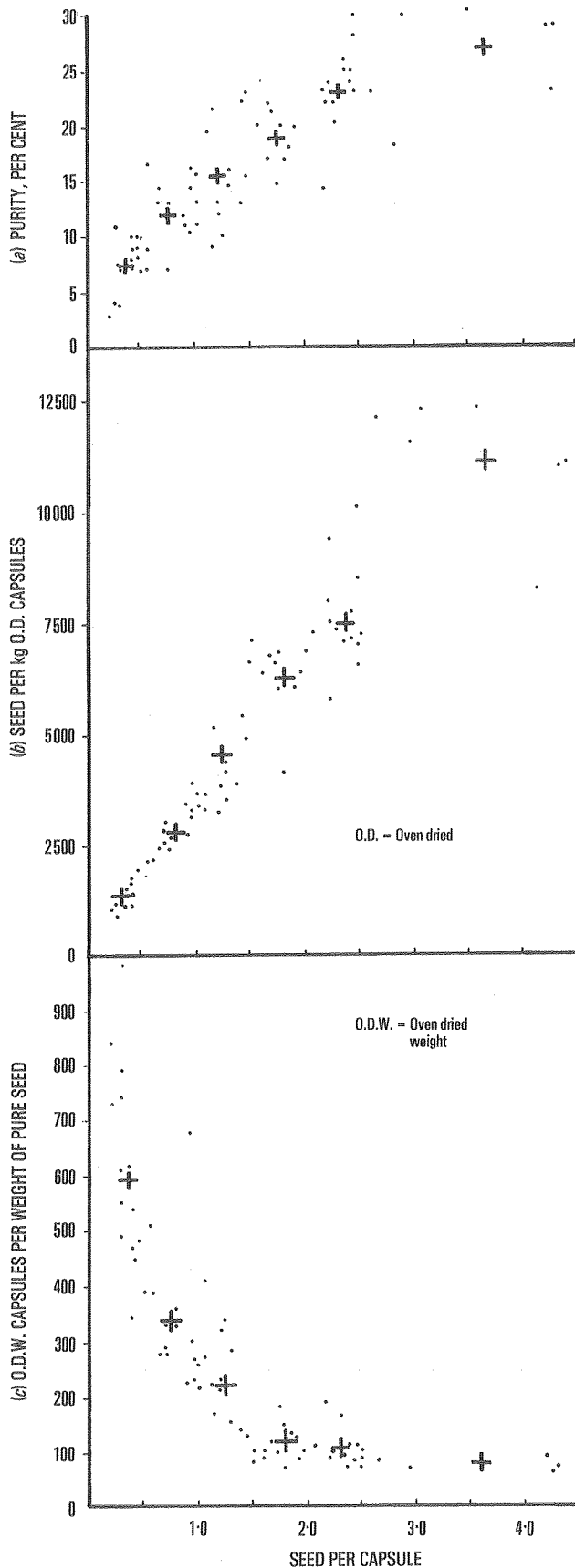


Figure 11

Seed per capsule related to effective yields. Crosses indicate group means.

Seed production

Seed production was high in 1967 and low to moderate from 1962 to 1964. During 1967 trees produced large quantities of seed (530 to 830 seeds per square metre) irrespective of their dominance class. In contrast, in 1964 when seed production was only moderate, dominant trees produced twice as much seed as sub-dominants (250 compared with 120 seeds per square metre, Table 6).

Costs of collection of capsules and seed

Twenty million seeds (27 kg pure) were collected during the 1967 seed crop. Ten tonnes of capsules were collected at two million capsules per tonne, averaging 1.0 seeds per capsule. The overall cost of \$24 for collection and extraction per 100 000 seeds was comparable with the average price of two parcels of seed purchased, at that time, from the Eastern States: \$32 for 110 000 seeds of *Eucalyptus muellerana* (55 000 seeds per kilogram) and \$16 for 110 000 seeds of *E. pilularis* (220 000 seeds per kilogram).

Due to great variation in the moisture content of capsules within and between seasons at any time, the fresh weight of a number of capsules varies greatly in comparison with their constant oven dry weight (= 100 per cent). Consequently numbers are difficult to assess by fresh weight. The weight of the moisture in the capsules in the winter (150 per cent) can be three times greater than the moisture content in the summer (50 per cent). Appendix I shows that fresh weight can be more than twice the oven-dry weight (for example, moisture content = 110 per cent). Volume measurement of a number of capsules, however, is more practical for operational purposes.

The relative cost of harvesting karri capsules in 1967 was \$55 per cubic metre (approximately 800 000 capsules) for combination manual and machine harvesting, and \$110 for manual harvesting. Adjusting for inflation, this is equivalent to about \$130 and \$260 respectively in 1977.

DISCUSSION

Seedbed preparation and the stimulation of seed dissemination are dependent upon fire. Therefore, a thorough knowledge of the seed production cycle is necessary to synchronise regeneration burning with seed maturity. Failure to achieve this may result in partial regeneration only, or complete failure to regenerate, thus necessitating immediate application of artificial regeneration methods to prevent weed infestation.

Preparation for regeneration begins with the demarcation of logging areas (Loneragan, 1961), and therefore advance planning is essential. This is possible only if the phenological patterns of karri are known and the potential seed harvest is reliably estimated. Only on this basis is it possible to decide whether collection of the seed crop for subsequent artificial regeneration is economically feasible (abundant crops only), whether natural regeneration can be relied upon (in years of both heavy and moderate seed crops), or whether artificial regeneration is necessary (in lean seed years). Close monitoring of the seed crop is particularly important one or two weeks before a proposed regeneration burn, or one or two days before seed collection.

PRACTICAL APPLICATION OF FINDINGS

Assessment of seed crops

The yield of seed can be estimated using the sampling procedures described earlier: the number of crown units is estimated visually and the number of floral components per unit is obtained by sampling.

The number of capsules in a crown can be assessed using a suitable telescope with a grid. The degree of crown cover can be measured using a densiometer (Lemmon, 1957) or assessed by vertical projection of crown cover along a line transect. Branch samples can be either shot down from standing trees or collected

Table 6
TREE SEED PRODUCTION IN 1964 AND 1967

Year	Crown class of 6 trees	Stem diameter (m)	Crown area (m ²)	Capsules		Seed yield (pure)			
				Number (n)	Capsule density (n/m ²)*	Weight (g)	Number (n)	Seed density (n/m ²)†	Number of seeds per capsule
1964	(1) dominant	1.19	298	34 000	114	77	76 000	255	2.2
	(2) subdominant	0.91	383	40 000	104	63	47 000	122	1.2
1967	(3) codominant	1.31	558	240 000	430	644	360 000	645	1.5
	(4) codominant	1.07	566	205 000	362	508	325 000	574	1.6
	(5) subdominant	0.70	48	20 000	417	68	40 000	833	2.0
	(6) subdominant	0.61	169	48 000	284	95	90 000	532	1.9

* Capsule density = number of capsules ÷ crown area

† Seed density = number of seeds ÷ crown area

from felled trees. An adequate sample is five to ten branches per crown. The samples can then be used to assess the average number of capsules per twig and the average number of seeds per capsule. From these basic data, taking thirty samples per population, the yield of seeds per tree, or per unit area of forest, can be estimated.

The floral components of different crown regions have different flowering seasons and therefore must be separated in the above samples and counted separately. The samples for each region should be thoroughly mixed and sub-sampled to determine the size, viability and vigour of the seeds from the different crops.

The known relationship between crown area and number of twigs during seed production (in karri there is an average of 21 twigs per square metre of crown area) is a useful abbreviation in the sampling procedure (Table 5).

The seed estimate can be kept up to date by repeated sampling during development and ripening of capsules. Losses during development are largely influenced by the age, vigour and order of the branchlets to which they are attached. The higher order branchlets die first and cast their seed in the first summer of the ripe crop. The vigorous main order branchlets continue leaf flush; green capsules, attached directly to these, are usually retained until early in the second summer, when most dry out and shed their seed.

During the first summer the number of twigs bearing mature to near-mature seed is halved to about ten twigs per square metre, and this decreases to one twig per square metre, or less, early in the second summer.

Two alternative methods are available for estimating the quantity of capsules needed for a given number of seed.

- (1) When sub-sampling for yield of seeds per capsule is being carried out, the weight of capsules required to yield 100 000 seeds equals

$$\frac{\text{weight of capsules sample}}{\text{number of viable seeds in the sample}} \times 100\,000$$
- (2) Seeds per capsule may be estimated by dissecting the capsules in the forest. Fig. 11 (b) can be used as a guide to estimating oven-dried (OD) capsule samples.

The estimated weight of fresh capsules per 100 000 seeds (allowing for variation in moisture content of capsules in the dry and wet periods) equals

$$\frac{70 \pm 20 \text{ kg}}{\text{number of seeds per capsule}}$$

Harvesting of capsules and extraction of seeds

So that the origin of the seed can always be traced seed collection was documented according to the rules adopted by the International Seed Testing Organisation in Dublin in May 1953, and Certificates of Seed Quality were prepared as recommended by the Food and Agriculture Organisation (Baldwin and Holmes, 1955).

Because of the great height of karri trees, capsules can only be collected when the trees or branches are felled. However, all seed may be shed within one or two days of the felling of the tree and prompt collection of capsules is therefore essential.

Seed collection is most efficient when the stands and their individual trees are assessed beforehand. For stands the primary concern is with the overall vigour and form of trees, the quantity of capsules and the number of seeds per capsule. For individual trees the accent is on dominance and form, though it is realised that these are only phenotypic expressions of survival and therefore genetic potential. The collection rate for an inexperienced harvester is about 10 000 capsules per man-hour.

For manually collected twigs seeds are extracted by heaping the twigs on tent flys or iron sheets for three sunny days, and then the chaff and seeds are shaken out. With machine harvesting, fruiting branches collected by hand are fed into a mobile tractor-driven harvester. The capsules are stripped from the branchlets, the leaves are blown away, heavier fragments are discarded by reciprocating mesh screens and the capsules fall through these into a chute and bag. Capsules are cured in a rotating, electrically driven and heated extraction kiln.

Fumigation and storage

All karri seed, whether intended for long- or short-term storage, should be placed in a closed container and fumigated with carbon disulphide at the rate of 5 ml per litre, for twenty-four hours, to minimise insect damage. The viability of karri seed stored in this way decreases very slowly (only 1 per cent per year for the first eight years of storage). High temperatures, high moisture content and frequent changes in storage conditions reduce viability. Seed intended for long-term storage should therefore be dried to 10 per cent moisture content and then stored at low humidity in a sealed container at 2 to 4°C. Even seed stored locally for shorter terms should be kept in sealed bags with silica gel, which serves as drying agent and moisture indicator, and stored in sealed bins at a cool, constant temperature. The bins should be insect- and vermin-proof.

Pollination Studies

INTRODUCTION

Sampling of floral components shed in karri stands, as described in earlier chapters, showed that from 70 to 90 per cent of the blossom was lost in immature fruit drop and that the total number of opercula exceeded the total number of mature seed by four to thirty times. The highest yield between 1957 and 1966 was in 1963 when, with an average yield of 1.5 seeds per capsule, 12 million flowers per hectare in stocked stands yielded 1.5 million seeds (Chapter II). A higher yield is desirable to increase seed supply and reduce regeneration costs.

The following methods, which have been used to increase seed supply in horticulture, were examined:

- (1) Introducing bees to improve pollination;
- (2) Partially ring-barking trees to improve fruit-setting;
- (3) Applying artificial pollination to increase the ratio of seed to capsule.

METHOD

Individual trees

The experimental trees were two mature karri trees, each with a ladder to the top for fire detection (Chapter I). Four cages were put on one tree (Diamond), two on the other (Gloucester). One of the cages, intended to house a colony of bees, was $2 \times 2 \times 3$ m. The remaining cages were smaller, $1 \times 1 \times 1$ m. All cages were constructed of wire and light wooden slats and were covered with open-weave cloth (Plate 5).

The distribution of the cages for various treatments is shown in Table 7.

The treatments were commenced in December 1965 at the beginning of the flowering season. A small colony of honey bees was introduced into the largest cage and, although self-pollination was encouraged, some cross-pollination was possible through a small entrance at the back of the hive which opened to the outside of the cage.



Plate 5

One of the cages used for pollination studies in Diamond Tree. Note the bee colony on the superstructure.

Bees and other pollinating insects were, as far as possible, excluded from the remaining five cages. The manual pollination within the cages followed Pryor's (1951) method:

Table 7
DISTRIBUTION OF POLLINATION TREATMENT*

Locality (identity of tree)	Pollination by bees		No treatment†	Artificial pollination	
	Without cage (open crown)	Inside cage with bees	Inside cage without bees	Inside cage	
				Self pollination	Cross pollination
Diamond Tree	Bees present	Cage 1	Cages 2, 3, and 4	Cage 3	Cage 4
Gloucester Tree	Bees present	No bees†	Cages 5 and 6	Cage 5	Cage 6

* Using numbered cages 1-6 in two trees

† Experimental "controls"

- (1) Emasculation of the flowers by cutting around and under the ring of stamens above the ovary;
- (2) Using a new paintbrush to dust fresh dry pollen on to the stigma.

Pollen for the artificial pollination was collected in advance. For self-pollination the pollen came from the same tree as the flowers being pollinated. For cross-pollination the pollen came from the other tree, that is, flowers on Diamond Tree were pollinated with pollen from Gloucester Tree and *vice versa*. Initial pollination was followed by a second pollination seven days later. All artificially pollinated flowers were marked.

A parallel set of treatments was enclosed in muslin bags to separate the effect of wind from the effect of bees. These treatments were a failure because the fine mesh trapped the air and moisture which caused the enclosed leaves and flowers to rot.

Development and duration of flowering and nectar flow were noted concurrently with the pollination studies. Coloured markers tied to branchlets were used to designate the time of flowering. Nectar content of flowers and nectar capacity of bees were measured to estimate how many flowers were necessary to match the forage capacity of bees. Just before flowering some buds on Diamond Tree were enclosed in a plastic bag which had pinholes to prevent condensation from transpiration, and nectar from flowers in this bag, and from others in the cages of both trees, was measured with a syringe graduated in 0.01 ml. The regurgitated nectar of twenty-five bees and the nectar capacity of twenty-five other bees were measured with the assistance of S. Chambers (Western Australian Department of Agriculture).

Whole stands

In the initial tests, honey-bee pollination and seed-tree sap restriction (as used in horticulture for increasing fruit crops) were investigated as means of increasing seed production. Before the trees blossomed in December 1965 a thirty-hive apiary with about 1.5 million bees was introduced into a cut-over 50 ha stand with 14 per cent crown cover. Four seed trees per hectare had been retained (Plate 6).

Partial sap restriction was begun 400 to 800 m from the apiary late in 1966. Eight trees were frill-ringed and eight were sap-ringed. Twenty trees were wire-banded but this was found unsatisfactory because either the breaking strain of the wire was too low, or the wire touched only the high spots around the irregularly shaped bole.

In January 1967 capsule production on standing trees was estimated using the telescope method. At the same time crown cover within the stand was estimated using a densiometer. Branch samples were then shot down from standing trees and the number of seeds per capsule was determined. The samples

came from treated (frilled) and untreated trees, located at various distances from the apiary during flowering. The maximum distance sampled was 3 km from the apiary. A regeneration burn was carried out in the area shortly afterwards and the seed dispersal was monitored using forty sampling trays.

RESULTS

Individual trees

The progress of flowering over one month (April-May) was as follows:

- (1) The operculum lifted six days after a white abscission line appeared between the operculum and the rim of the hypanthium.
- (2) The operculum was shed exposing a greenish-yellow unripe stigma and stamens with anthers fully curved.
- (3) The stamens lifted exposing the anthers; in four to five days the pollen became powdery and the stigma ripened.
- (4) The nectar flowed for five to eight days, depending on the weather.
- (5) The anthers turned brown and then the stamens withered and were shed within one week.
- (6) The floral disc dried up over the next one to two weeks.

The quantity of nectar [Item (4) above] averaged 0.04 ml and varied from nil to a maximum of 0.09 ml per hypanthium. The capacity of the flower cup was about 0.05 ml. The body capacity of the bee to hold nectar was 0.045 ml, of which 0.033 ml was regurgitated.

The capsules from the pollination experiments were sampled at two stages:

- (1) Nine- to twelve-month-old capsules from the April-May pollination.
- (2) Eight-month-old capsules from the June pollination.

The first stage corresponded to the peak of flowering in Gloucester Tree, the second to the peak of flowering in Diamond Tree.

Capsules from the April-May blossom were slower to ripen than those from the June blossom. Robust capsules on vigorous branches ripened more rapidly than the capsules on weak branchlets.

Some caged and uncaged branches on Diamond Tree died during the experiment; the pollen cross from Gloucester Tree and the bees-in-cage treatment were among these. Nevertheless, the last forty-eight green capsules from this bee-pollinated branch yielded 1.98 seeds per capsule nine months after flowering. This was nearly twice that from flowers from which bees had been excluded, and approximately 25 per cent higher than from flowers in the surrounding open crown (Table 8). It was also superior to yield



Plate 6

A thirty-hive apiary at West Manjimup showing clear felling with seed trees.

Table 8

MEAN SEEDS PER CAPSULE (s/c) IN POLLINATION EXPERIMENTS

<i>Source</i>	<i>Bee pollination</i>			<i>Artificial pollination</i>	
	<i>Cages without bees</i>	<i>Cage with bees</i>	<i>Open crowns</i>	<i>Self</i>	<i>Cross</i>
From capsules 9 to 12 months old					
(1) Diamond Tree (s/c)	1.08	1.98	1.60	1.32	—
(2) Gloucester Tree (s/c)	1.23	—	1.94	—	4.03
From capsules 8 months old					
(1) Diamond Tree (June blossom, s/c)	0.85	1.15	1.11	0.67	0.96

per capsule from caged, self-pollinated flowers. The yield from Diamond Tree appeared to be somewhat better overall for the April-May pollination, but poorer for the June pollination; the cold, wet June weather probably reduced the activity of bees, pollination and seed development. The most outstanding yield resulted from manual cross-pollination on Gloucester Tree in April-May (4.03 seeds per capsule), which is above any reported natural yield (Table 8).

Whole stands

As frilling did not appear to affect the seed yield per capsule, data for both treated and untreated trees were pooled to obtain a better basis on which to assess the effect of distance from apiary. The results are shown in Table 9. The average yield of seeds per capsule was 50 per cent higher for the eight trees near the apiary than for the twenty-eight trees 480 to 800 m distant (1.99 compared with 1.25).

With increasing distance from the apiary the yield of seed per capsule dropped still further. The average yields from three sets of samples in a medium quality karri-marri stand 2 to 3 km from the apiary were 0.9, 0.9 and 1.0 seeds per capsule respectively. In a high quality karri-marri stand 2 to 3 km from the apiary, the average yields from three sets of samples were only 0.4, 0.3 and 0.8 seeds per capsule respectively. However, part of this poor yield was due to immaturity of the capsules.

Nevertheless, it is possible to generalise that proximity to the apiary influenced the yield of seed per capsule, indicating that the bees were more active in pure karri stands near the apiary than in mixed stands further away.

Sampling with trays did not allow comparison of seed shed from individual trees. It did, however, provide a useful confirmation that the yield from the stand, in terms of seed collected in seed trays after a regeneration burn, is comparable to the calculated yield based on prior assessment of crown cover, number of capsules per unit area of crown, and number of seeds per capsule.

DISCUSSION

Bees significantly improved seed yield per capsule near the apiary site in the karri stand, but their range of effectiveness was not adequately tested. Studies comparing the seed yield from open crowns with the yield from caged branches also showed that bees increased the yield considerably. This suggests that bees could play an important part in improving the effectiveness of karri seed collection, as the low number of seeds per capsule is probably the largest single factor influencing the cost of harvesting.

The relationship between bees and karri is mutually beneficial, since karri forest is one of the best sources of honey in Western Australia. Apiaries of 200 hives and 12 million bees yielded 45 kg of honey per hive

Table 9
SEED APPRAISAL AT GRAY BLOCK, 1967

Conditions	Block treatment								Total		
	Untreated				Wire banded		Ringbarked		Under trees	In gaps	Means
	Near apiary (S.E. *)	Near apiary (N.W. *)	Group 1 (N.W. *)	Group 2 (N.W. *)	Group 1 (N.W. *)	Group 2 (N.W. *)	Sap rung (N.W. *)	Frill rung (N.W. *)			
Distance from apiary (m)	60-100	0-120	650	800	525	575	700	800	—	—	—
Seed per capsule	1.99 (a)	1.99 (a)	1.25 (b)	1.25 (b)	1.25 (b)	1.25 (b)	1.25 (b)	1.25 (b)	—	—	1.51
Number of trees	4	4	4	4	4	8	4	4	36	—	—
Number of trays	7	10	2	2	2	7	1	2	33	9	—
									Means		
Estimated capsules (n/m ²)	420 (c)	469	638	504	650	685	588	672	—	—	578
Crown cover (%)	16.3	16.6	16.5	20.0	29.0	17.3	17.0	13.5	18.3	10.2	14.25
Crown cover (m ² /ha)	1630	1660	1650	2000	2900	1730	1700	1350	1830	1020	1425
Calculated seed ('000/ha)	1360 (d)	1550	1320	1260	2360	1480	1250	1130	1600†	890	1240
Arithmetic means	—	—	—	—	—	—	—	—	1460	—	1175
Actual seed ('000/ha)	1000	2000	1320	2290	2470	1730	1090	1640	1690	1280	1485

NOTE: * N.W. = North-west of apiary. S.E. = South-east of apiary.

(a) Seed per capsule: $4015 \text{ seeds} \div 2021 \text{ capsules}$, viz. (a) = 1.99
 (b) Seed per capsule: $4548 \text{ seeds} \div 3650 \text{ capsules}$, viz. (b) = 1.25 } mean = $8563/5671 = 1.51$

(c) Estimated capsules, see Appendix II.

(d) Calculated seed per hectare for crown cover: $\left\{ \begin{array}{l} \text{m}^2/\text{ha} \times (c) \times (a) \\ \text{m}^2/\text{ha} \times (c) \times (b) \end{array} \right\}$ calculated means $\left\{ \begin{array}{l} 1830 \times 578 \times 1.51 = 1600000^\dagger \\ 1020 \times 578 \times 1.51 = 890000 \\ 1425 \times 578 \times 1.51 = 1240000 \end{array} \right.$

each month during the prime flowering season between November 1965 and March 1966. This high daily honey yield per hive of 1.5 kg [815 ml per kilogram for specific gravity 1.45 and 85 per cent combined sugars (glucose) derived from 1850 ml per kilogram nectar, of specific gravity 1.20 and 45 per cent glucose (Smith, 1966; Wedmore, 1955)] can be equated with the number of karri flowers on the basis of the known yield of nectar per flower. Assuming the average daily yield of nectar per flower to be 0.04 ml, 70000 flowers ($1.5 \times 1850 \times 25$) are needed to supply one hive for one day. This amounts to seven million flowers for the above hundred-day flowering season which corresponds to about two hives per hectare.

Under existing bee-keeping regulations, apiary sites in State Forests are spaced on a 3×3 km grid, that is, one apiary per 900 ha. An important consideration in specifying this spacing is the transfer of diseases between apiaries. If foraging by bees were independent of distance then each apiary site could support 1800 hives in a heavy flowering season. No apiaries of that size exist, although 600 hives are known to have been established at one site. Competition for flowers between apiaries would be minimal under these conditions, as even 1.5 km is too far for efficient honey foraging. This means that higher yields from seed crops can be expected near apiary sites and this knowledge should be used to plan karri seed harvest.

In years of moderate flowering the area required to support a hive would probably need to be increased to 6 or 7 ha. In poor years no less than 20 ha may be needed. However, hives would be widely dispersed and this would no longer be attractive to bee-keepers.

Inadequate fertilisation of flowers is only one of the factors contributing to the poor seed production in karri. As discussed earlier, there is continual abortion of developing floral parts (buds, flowers and capsules). Much of this can be attributed to insect damage. In 1960 weevils (similar to *Haplonyx tibialis* associated with *E. gomphocephala*) nipped off 1600 buds per square metre of crown area, virtually exterminating the crop. In 1960 an attack by larvae of borers (*Bruchidae*) in karri capsules reduced the number of viable seeds per kilogram of seed and chaff to 37000, that is one-third of the long-term average. These two pests are not the only cause of karri seed loss. Other insect larvae have also been observed in capsules and seeds.

PRACTICAL IMPLICATIONS

As no practical, economically viable treatment to prevent these losses has so far been devised, it is particularly important to make maximum use of any good crop. In particular, the seed that is surplus to natural regeneration requirements in years of heavy seed production should be harvested and stored for artificial regeneration in lean years.

The effect of apiaries on seed production in adjacent forests needs further validation. If the findings reported here are confirmed and reinforced, granting of apiary sites to bee-keepers should be based on seed production requirements, and bee-keepers should be encouraged, by waiving of fees and granting of subsidies, to distribute their hives at apiary sites in key areas. In view of the importance of seed production to karri, and in view of the high cost of seed harvesting, these steps would be fully justified.

Factors Influencing Flowering and Seed Production

INTRODUCTION

Although there is considerable predictability in karri seed production, records over the past forty years disclose two decades in which this predictability was unreliable. One of these was the decade commencing 1938, recorded by Forester M. J. O'Sullivan, and the other was the decade commencing 1957, recorded by O. W. Loneragan (Table 10).

A reasonably reliable record of the location and timing of abundant karri flowering and seeding now dates back to 1925 in the West Pemberton district. This record is connected with log harvesting and regeneration burning, and the resulting regenerated stands provide evidence that heavy seeding has occurred. A summary of flowering and seeding patterns from 1925 to 1971 is shown in Table 10. This

Table 10
PHENOLOGY OF FLORAL COMPONENTS FROM 1925 TO 1971

Formation of flower bud initials	Blossom	Seed years (a)				
		Main	Subsidiary		Local	Seed none to rare
Year	Year	+ 8	+ 4 (Major)	0 (Minor)	—2	—4
1925 (b)	Not recorded	1929	1930	—	1925, 1928	1926, 1927
1930	1931, 1932 (c)	1933	—	1934	1935	1931, 1932
1934	1935, 1936	1937	—	1938	—	1936
1938 (b)	1939, 1940 , 1941	—	1942	—	1941, 1943	1939, 1940
1943	1944 , 1945	—	1946	—	1945, 1947	1944
1947	1948, 1949 , 1950	1950	1951	1949	1952	1948
1952 (b)	1953, 1954	1956	1957	—	—	1953, 1954, 1955
1956	1958	—	—	—	1960, 1961	1958, 1959
1960	1960, 1961 , 1962, 1963	—	1962	1963, 1964	1965, 1966	—
1964	1964, 1965-1966 , 1967	1967	1968	—	1969	1970, 1971

(a) The scale was designed to give a quantitative assessment of the seed crops (Fig. 12).

1. Main—karri forest region
2. Subsidiary—river valley system, subdivided:
 - 2.1. Major
 - 2.2. Minor
3. Local—local stand only.

(b) The inflorescence formation in these years was not recorded. Assuming a four-year cycle to seed, these dates are taken from the appropriate seed years.

(c) The years of heaviest blossom are shown in bold figures.

Relative importance of seed crops has been expressed by relative numbers according to an arbitrary scale which makes quantitative analysis possible (Fig. 13).

early evidence can now be related to the floral cycles described in Chapters I and II.

Adequate seed production for the naturally regenerated stands usually recurred in cycles of four to five years, though not necessarily in the same stands. Usually two or more years of adequate seed production for regeneration have been followed by two to four years without seed in most stands. Generally, spring and summer blossom has been followed by seed shed one year later; seed shed from autumn and winter flowering usually occurred two years later.

Observers from the Forests Department and the Department of Agriculture have suggested that soil moisture influenced both the flowering and the seeding of karri, but this was not tested quantitatively.

METHOD

To test the association of the intensity of karri flowering with soil moisture the relationship between them was examined graphically and mathematically. Cumulative rather than annual parameters have been used because:

- (1) soil moisture in any one year is influenced by the rainfall of preceding years; and
- (2) development and dispersal of karri seed crops is frequently spread over several years.

Rainfall records from various climatic stations in the karri region were totalled and averaged to provide a compound index of rainfall rather than simple parameters. The cumulation uses as the base line the year 1917, when the annual rainfall for the key station was 1900 mm, which is 50 per cent more than the long-term average. It can be assumed that soil moisture storage was at a peak that year.

As previous descriptions of seed crops were relative rather than quantitative, an arbitrary scale, ranging from +8 for a heavy seed crop to -4 for seed failure, was constructed to make quantification possible (Table 10).

RESULTS

The relationship between the cumulative seed crop and the cumulative deviation from annual rainfall is shown in Fig. 12.

Peaks and troughs in the rainfall index are followed, a few years later, by corresponding peaks and troughs in the seed crop index.

In quantitative terms, positive cumulative deviation from average rainfall is highly significantly correlated with cumulative seed crop one year later ($r = 0.50$). This interval corresponds to the time lapse between flowering and seed maturity. Positive cumulative

deviation from average rainfall is also highly significantly correlated ($r = 0.49$) with the cumulative seed crop four years later, which corresponds to the time lapse between the appearance of inflorescence buds and seed maturity (Fig. 13).

In contrast, there was no significant correlation between current (that is, non-cumulative) parameters of departure from average rainfall and seed crop. The highest, but still non-significant, correlation coefficient obtained ($r = 0.24$) was between annual rainfall and seed production one year later.

DISCUSSION

The patterns shown in Fig. 12 indicate that regular seed cycles can be expected only when soil moisture storage is adequate, as indicated by positive cumulative deviation from average annual rainfall. When it is not, seed crops become irregular and unpredictable. The former was the case for regular seed years between 1925 and 1937, the latter for unpredictable blossom and seed production between 1938 and 1947 and again between 1957 and 1964. Major droughts, such as those of 1940, 1959 and 1969, when rainfall was 25 to 30 per cent below the long-term average, appear to cause serious disruptions in the seed cycles, the effects of which are apparent for several years.

Conversely, initiation of heavy bud, flower and capsule crops is usually associated with spring-summer rainfall 40 per cent above the average. Furthermore, when soil moisture status is high, severe wildfires causing loss of foliage but low soil moisture depletion and then rapid uptake with crown recovery, may also initiate bud formation as occurred in 1937 and 1950.

In periods of irregular flowering cycles the prediction of seed crops is even more difficult than the prediction of flower crops. This is because there is an increased likelihood of bud, flower or immature fruit loss during these periods.

Flowering and seed production vary in individual trees owing to the complex interaction of heredity and environment. Although the basic cycle from bud inception to seed supply takes four years this does not mean that blossom and seed crop will appear regularly every four years. Initiation of bud crops and their development into seed crops is subject to the influence of climate, fire and biological factors, and is therefore difficult to predict.

Within this broad pattern of periodicity of the climate and phenological behaviour of karri, the duration of development of the annual shoots and initiation of floral buds varies in individual trees. There are also individual differences, apparently genetically based, in the frequency and seasonality of flowering. However, the need to replace food reserves exhausted by heavy flowering and fruiting is probably common to all trees.

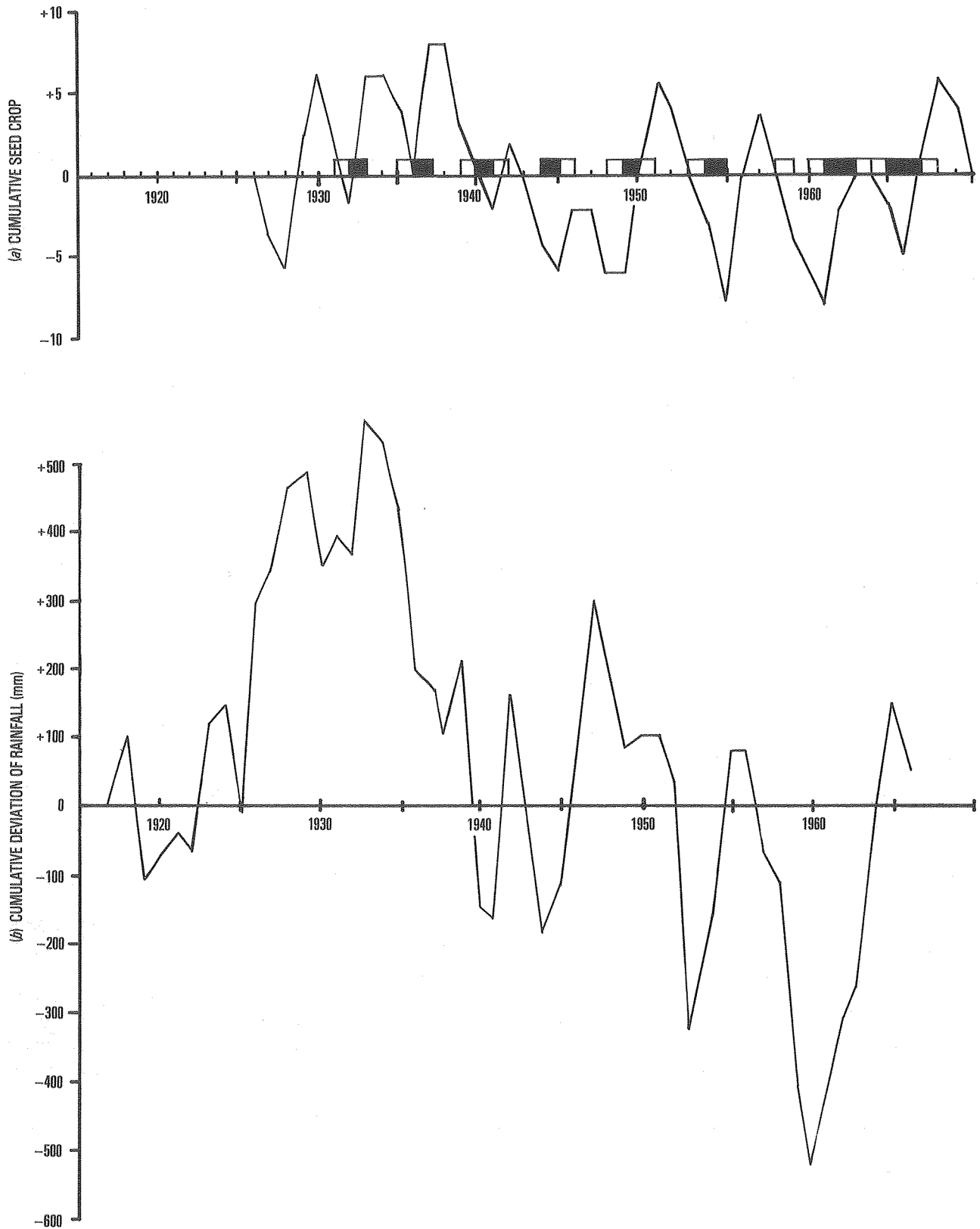


Figure 12

- (a) Cumulative seed crop. The plot was made using the scale for Table 10: the black squares show good honey blossom years; white squares, other blossom years.
 (b) Cumulative deviation of rainfall in the karri forest.

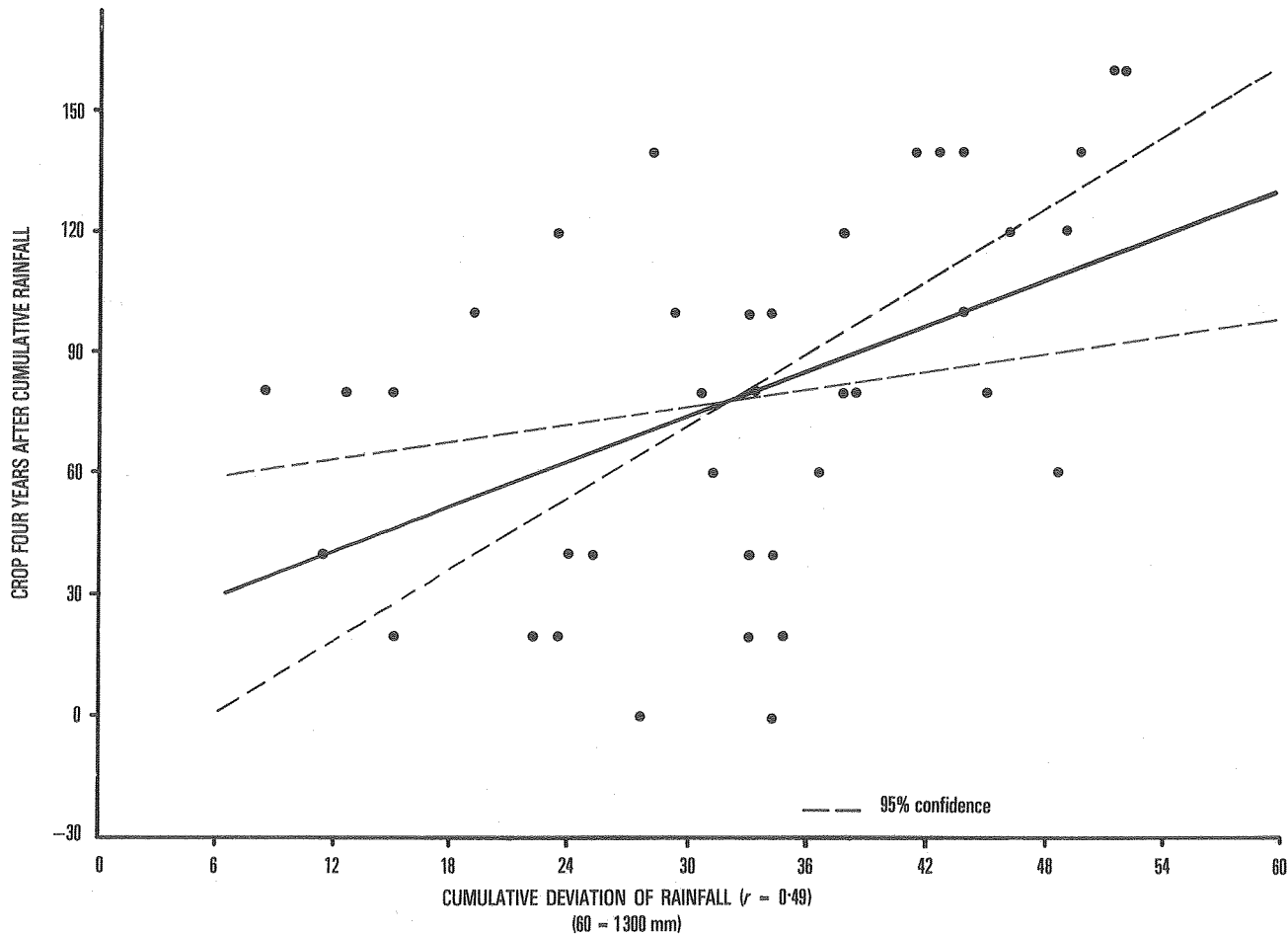


Figure 13
Correlation for Fig. 12.

Temperature undoubtedly exerts influence on rate of floral and seed development, which however, is complicated by the physiological requirements of consecutive annual bud crops formed during a long floral cycle. Nutritional requirements change and the hormone activity of one crop may slow down or speed up the development of another.

PRACTICAL IMPLICATIONS

Although cumulative deviation from average rainfall can be used as an index of likely heavy bud formation, it has to be borne in mind that a complex

set of other physical and biological factors also influences the production of a good seed crop. Furthermore, the rainfall itself is a variable factor.

In view of uncertain weather conditions during long-term observations it should never be assumed that a heavy seed crop will be followed by another heavy seed crop within a decade. Each heavy seed crop should therefore be utilised to the maximum, and adequate quantities of seed should be stored to ensure that all regeneration commitments for the following decade can be met.

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APPENDIX I

Seed Production and Yield

Example of measurements in production of capsules and seed yield of karri

FIELD COLLECTION	TREE No.	YEAR	14/67
Stem diameter			= 1.3 m
Crown area			= 560 m ²
Gross yield of seed extracted			= 2.236 kg
Purity = 23.8%			Net = 0.533 kg
<i>Weight per 1000 viable seeds (pure seed)</i>			= 1.74 g
Total viable seed collected			= 306000
<i>Number of seed per 20 g (with chaff)</i>			= 2740
Estimated percentage of seedcrop collected			= 90%
Subsequent completeness of extraction (after collection is completed) and checked by constant oven dry weight treatment at 105°C for 3 days			= 95.6%
LABORATORY TEST DATA (BEFORE FIELD COLLECTION)	Weight (g)	Number (n)	
Fresh capsules	800	1634	
Oven-dry capsules (6 days at 40°C)	380	1634	
Subsequent extraction (3 days at 105°C) = 15 seeds	—	—	
Pure seed extracted from sample (= a)	4.15	2524	
Chaff impurities (= b)	20.23	—	
Seed purity [$a/(a + b) \times 100$] = 17%	—	—	
Seed viability (percentage) = 98%	—	—	
CALCULATIONS			
Yield ratios			
(i) <i>By weight</i>			
Oven-dry capsules per seed $(380/4.15) = 92$	—	—	
Fresh capsules per seed $(800/4.15) = 193$	—	—	
(ii) <i>By number</i>			
Seed per capsule $(2524/1634) = 1.54$	—	—	
Total production in the crown (Example of calculations from yield ratios)			
<i>Sound seed</i> (before viability test):			
By weight = $0.533 \text{ (kg)} \times (100/90 \times 100/95.6)$	619	—	
By number = $306000 \times (100/90 \times 100/95.6)$	—	356000	
<i>Capsules</i>			
By weight = 619×193	119×10^3	—	
By number = $1634 \times 1000/800 \text{ (kg)} \times 119$	—	243000	
By number from seeds $356000/1.54$	—	231000	
Standard production per m² (Multiplying above by 1/560 for crown area)			
<i>Seeds</i>	1.1	640	
<i>Capsules</i>	210	420	

APPENDIX II

Telescope Calibration and Evaluation
for Appraisal of Fruiting

<i>Evaluation by telescope</i>		
<i>Elevation of sight (degrees)</i>	<i>Horizontal base line (m)</i>	<i>Vertical height to capsules at one-third crown depth from tree top (m)</i>
35	58.5	41
36	58	42
37	57	43
38	56.5	44
39	56	45
40	55	46
41	54	47
42	53	48
43	52.5	49
44	51.5	50
45	51	50.5
46	50	51.5
47	49	52.5
48	48	53
49	47	54
50	46	55
51	45	56
52	44	56.5
53	43	57
54	42	58
55	41	58.5
56	40	49.5
57	39	60

Heights for counting capsules at one-third crown depth from the tree top using telescope having $\times 30$ magnification lens, with standard grid of nine cells. Vertical area of cover = 0.1 m^2 at 71.6 m , constant length of line of sight.

The counted number of crown components in seven readings represents 1 m^2 of area in the horizontal plane. The crown area of 1 m^2 (Table 4) is equivalent to 0.7 m^2 through the telescope using this table (see text).

APPENDIX III

Seed Testing Procedures

SEED GERMINATION RECORD						
GENUS			SERIAL No.			
SPECIES			DATE			
PROVENANCE (origin)						
LEADER SAMPLES (under squash test)	Leader (l)	Weight (g)	Whole seeds (w)		Sound seeds (N ₂)	Percentage sound seed (all tests)
Calculations and size of test replicate =* seeds per replicate (\bar{n}_r) Mean weight (\bar{g}_r) = $\bar{n}_r \times G/N_2$ =	1					Calculate percentage sound seed $S = N_2/N_1 \times 100$
	2					
	3					
	4					
	5					
	6					
Totals	L =	G =	G ₁ =	N ₁ =	N ₂ =	S (%) =
Design of trial—show number of levels (n) for each factor (f)						
Factors		Levels (n) for (f)			Remarks	
F ₁ Pre-treatments F ₂ Temperature units F ₃ Replicates (R)					(State detail below †) L ₁°C; L ₂°C Identical (n) for each (f)	
Total number of cells (C) =; i.e. multiply (n) for each (f) above						
*Use 600 sound seeds when possible (e.g. $\bar{n}_r = 600/C$)						
Total sound seeds in test, number (C × \bar{n}_r) =						
weight (C × \bar{g}) =						
						List of Weights (g)
Pre-treatment†		Dates		Duration		
		Started	Ended	Days	Hours	
Soak						
Stratification (after soak)						
Other (show detail)						
Refer to "t Tables" for $t_{(n-1)}$ values [Fisher, R. A., and Yates, F. (1963). Statistical Tables, 6th ed., Oliver and Boyd, Edinburgh], for confidence limits $P = 0.05$.						
Calculate						
(i) Standard Error (SE) for data, not comparing treatments;						
(ii) Standard Error of Difference of Means (SED) for testing difference between means;						
(iii) $SE \times t_{(n-1)}$ for significant treatment (ii); or pool data for non-significant difference between treatments (i);						
(iv) Range in confidence limits (\pm) at $P = 0.05$ for SE of significant treatment (ii) or of all data (i).						
Calculations						
(i) For data not comparing treatments or for pooled data: the mean $\bar{x} = \frac{\sum X}{n}$ =; the						
$SE = \sqrt{\frac{\sum X^2 - (\sum X)^2}{n(n-1)}}$ =; the range (\pm) at $P = 0.05 = SE \times t_{(n-1)}$ =						
(ii) For testing difference between means for significant treatment, the actual difference between means must be greater than or equal to $SED \times t_{(n-1)}$, i.e. (iii); the mean, $\bar{x}_1 =$; $\bar{x}_2 =$; the actual difference ($\bar{x}_1 - \bar{x}_2$) =; the $SED = \sqrt{\frac{SE_1^2 + SE_2^2}{n(n-1)}}$; $SE_1 = \sqrt{\dots}$; $SE_2 = \sqrt{\dots}$;						
(iii) The difference for significant treatment at $P = 0.05 = SED \times t_{(n-1) + (n-1)}$ =						
(iv) Calculate range in confidence limits (i) for stated treatments.						
SUMMARY OF RESULTS (Describe significant treatment or state non-significant.)						
Treatment, t =						
Total seeds in the stated treatment (t) =						
Replicates (R) in treatment	Sound seed in treatment		Germination of sound seed [for Energy (%) at 4, 7, or 14 days]*			Germination (%) of whole seeds $N_1 =$
Totals $R_t =$	$\bar{g}_r.R_t =$	$\bar{n}_r.R_t =$	Total (N_2) =	$(N_2/\bar{n}_r.R_t) \times 100 =$ Capacity at 28 days (V) =%	$(S) \times (V)$ 100% (W)
$SE =$ $t_{(R-1)} =$			*Specify actual interval. Energy at days =% (E) Vigour (E/V) = 0.			
$SE \times t_{(R-1)} =$						
VIALE SEED (for above treatment)						
Grams per 1000 viable seed (pure) = $\left(\frac{1000}{N_2} \times \bar{g}_r.R_t\right) =$ g			For $P = 0.05$, range % = $\frac{SE \times t_{(n-1)} \times 100}{\text{mean } (N_2/R_t)}$			
Germinants per kg (with chaff) = $\left(\frac{N_2}{\bar{g}_r.R_t} \times 1000\right) =$						
Confidence limits; number per kg ($P = 0.05$) = (above × percentage range) = \pm						
Range in number of germinants per kg = to						

APPENDIX IV

Seed Sampling Procedures and Method of Summary

1. Weigh seed (by sub-sample method), squash test 6 sub-samples, count seed, take a mean of 6.
 2. Weigh 6-12 replicates.
 3. Soak them overnight (or as stated for pre-treatment).
 4. Dry them on filter paper and dust them with T.M.T.D. (fungicide).
 5. Put into petri dishes and number them.
 6. Count and water regularly (maintain surplus condensate).
4. Dry them on both filter paper and blotting paper, then put into a screw top jar with T.M.T.D. and shake.
N.B.—Use standard safety precautions.
 5. Petri dishes should be three-quarters filled with vermiculite and wetted first. Lay filter paper over vermiculite and evenly spread treated seed over. Label each container and put into cabinet at the required temperature.

SUB-SAMPLE METHOD—LARGE SAMPLES

Use standard sampling tubes or dividers.

SUB-SAMPLE METHOD—SMALL SAMPLES

1. Tip seeds on to a flat plastic sheet. Mix well, divide into four quarters, take opposite quarters and put them aside. Take remaining quarters and mix them together well. Quarter again, and again put aside the opposite quarters. Continue this to leave approximately 25-30 seeds in sub-sample. Squash and count the seed (good seeds have an oily texture and mark the paper when squashed). Repeat this procedure SIX times (Appendix III).
The weights are then added up and divided by the number of seed (this gives the weight per seed). Multiply this weight by number of seeds per petri dish (e.g. usually $50 \times 12 = 600$) for each test.
2. Weigh out 12 samples of seed by sub-sample method.
3. Put the samples into plastic tubes and give the pre-treatment needed, i.e. overnight soak, stratification or boiling water.

ENUMERATION OF GERMINANTS

Check regularly for continuous water condensation in dishes and count at 4, 7, 14 and 28 days.

SUMMARISE

Serial number
Origin (Appendix V)
Blossom time
Kilograms collected (Appendix VI)
Grams extracted (seed with chaff)
Viable seed per 20 g seed with chaff (Tz method*)
Weight per 1000 seeds (pure, without chaff)
Germinants per 20 g (seed with chaff) (dish method)
Germination medium used and conditions of use (temperature, moisture)

* Tetrazolium chloride method

APPENDIX V

Seed Origin Card Supporting Seed Report (Appendix VI)

Seed Origin Record	
(Seed Capsule Sample 0.5 kg)	KC1 Card No.
Add "PLUS" for Superior Tree	District
Soil type	Topographic position
Block	Aspect Slope (degrees)
Landing No.	Quarter Map Ref.
POSITION OF SAMPLE AND FREQUENCY OF BUDS, BLOSSOM AND FRUIT	
In leaves (immature)	
At base of leaves (fresh mature)	
Well behind leaves (old mature)	
(N.B.—Keep different crops in separate samples)	
Number of trees sampled	
Species	

(Front of card)

DETAILS OF TREES SAMPLED FOR SEED COLLECTION	
Tree class	Canopy class Vigour
Tree girths	Crown diameter
Tree heights	Bole lengths
Branching angle	diameter length
Branching frequency, shape of bole	
Collected by	Date
SEND TO REGENERATION CO-ORDINATOR OR SILVICULTURIST	

(Back of card)

Test Sampling for Fruit Collection and Yield Results

SEED REPORT										Net Weight	
Seed Serial No. Date										Fruit	Seed
Collection Card No. from (district)											
Species (correct sp.) Origin											
Date collected Date received Gross weight received (kg)											
Sampling and treatments on arrival											
..... Net weight after treatment											
Ratios of { Fresh fruit/pure seed Temperature °C Number of days Date started a.m. Ended a.m.											
Weight { Oven-dry fruit/pure seed Temperature 105°C Number of hours Date started p.m. Ended p.m.											
Sample Test No.											
Calculated to—	Weight in sample (g)			Convert	Number in sample			Converted to		Weight required to collect 100 000 seeds	
Sample components	A	B	C	($\frac{3}{5}$) × 100	A	B	C	Total	No. per g	No. per kg	(First column × 100)
(1) Fresh fruit											(1) kg
(2) Oven-dry fruit											(2) kg
(3) Pure seed				=%							
(4) Impurities											
(5) Seed and impurities											
Weight per 1000 viable seed (last column ÷ 100)				(6) Number of seed/fruit				(a)	Incomplete extraction		
				(7) Extra seed at O.D.W.				(b)	(b)/(a + b) × 100 =%		
(5) Seed and impurities				(8) Sound seed per 100 cut				(9) Sound seed per single fruit			
(2) Oven-dry fruit				(10) Chemical coloured seed Tetrazolium chloride (Tz) method				Viability (percentage) =% (strong + weak)			
(1) Fresh fruit				Red				} Strong seed =% Weak seed =%			
				Bright pink							
				Pale pink							
			White				= Dead				