SOME ASPECTS OF REPRODUCTIVE BEHAVIOUR IN *EUCALYPTUS GRANDIS* (HILL) MAIDEN

by

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A corner of the *E. grandis* seed orchard at Zomerkomst, with a plantation in the background.

The plantation has been raised from seed derived from open-pollination in an isolated seed orchard. It has been planted so as to act as a screen to protect the seed orchard shown above against contamination by pollen from neighbouring plantations.

Most of the ramets to be seen in the seed orchard have been cut at a height of two metres and the branches have been weighted down or cut back, to arrange for convenience of access to flowers and fruits. The few ramets which have not been cut in this way are being given other treatments as part of a test to find the best seed orchard management practice for seed production and collection.

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EXPLANATION OF SOME TERMS USED

Selfing

This term is used for self-pollination between flowers of two or more ramets within a clone and between flowers of a single ramet, as well as within individual flowers.

Self and cross

These terms are used as abbreviations in place of expressions such as "the progeny from self-pollination treatments" and "the progeny from cross-pollination treatments" respectively. The abbreviations are made where required to avoid tedious repetition.

Self pollen, cross pollen and mixed pollen

In any one test, these terms are used in place of "the pollen used in self-pollination", "the pollen used in cross-pollination" and "the pollen used in mixed - (self and cross) - pollination" respectively. This is mainly done in connection with tests of selective fertilisation in which competition between pollen from different sources is investigated.

Mature buds

Flower buds are referred to as being mature when the inner operculum starts to split away from the calycine ring. It is the stage at which buds are most commonly emasculated and the stage is normally followed within a few hours by anthesis.

Chapter 1

INTRODUCTION

The fast rate of growth and the timber properties of *E. grandis,* when grown on suitable sites, make it useful for a wide variety of purposes. At an early stage in the history of South Africa, it was used for poles and mining timber; later its potentialities as saw timber became recognised especially for box shooks, furniture and flooring. More recently there has been an increased demand by paper and fibre board manufacturers and by the cellulose manufacturing industry (van Laar, 1961).

Forest Department statistics as at 31/3/73 show that plantations of the species cover an area of 275 000 hectares and the timber provides a significant proportion of the hardwood requirements of the country.

Under local conditions, there is much loss of timber from degrade on sawing *E. grandis* logs, i.e. from splitting and other defects, and there is room for improvement in bole straightness. In 1964, the Government Forestry Department opened a tree breeding station at Zomerkomst, near Tzaneen in the Northern Transvaal. The main object at this station was the improvement of timber quality, the procedure adopted being that of recurrent selection (Shelbourne, 1969).

Known breeding work being done elsewhere with eucalypts includes that with *E. grandis* in New South Wales and Zambia (Burgess, 1973; Christensen, 1973), with *E. robusta* in South Florida (Franklin & Meskimen, 1973) and with *E. delegatensis* and other species in Australia and New Zealand (Eldridge, 1973).

Such programmes entail selection of trees as a first step, followed by their propagation by one means or another. At Zomerkomst, selected trees are propagated by grafting, using a method similar to the "rind graft" described by Garner (1958). The grafted plants are established in a seed orchard, also in a tree bank. The former area is for the production of bulk seed for afforestation using a site which is isolated from other pollen sources capable of causing contamination. Each selected tree is represented by several ramets, or individual grafted plants, known collectively as a clone; there may eventually be 100 or more of these clones in the orchard. The individual members of each clone are scattered throughout the seed orchard with a view to the promotion of cross-pollination between clones.

The tree bank on the other hand is not for seed production, but rather serves as a store for clonal material, including that which is not immediately required. Normally, only one or two ramets of each clone are planted in it.

As in any other plant breeding undertaking, flower phenology and morphology are necessary objects of study, as are also aspects of floral mechanism, pollination, vectors and receptivity. When these have been studied, the way becomes clear for investigating the breeding system of the species.

Since the aim is to produce improved seed, it is necessary to know whether the clones are self-fertile and the frequency of production of selfed seed under seed orchard conditions. If the latter is appreciable in any of the clones, then the nature and extent of any inbreeding depression would need to be investigated and it might become necessary to find some means of limiting the production of inbred progeny in the seed orchard. It is also necessary to investigate any barrier to self-fertilisation which may exist in nature.

Therefore, the main object of the study described in this thesis was to investigate certain aspects of the breeding system of *E. grandis,* mainly by applying various controlled pollination treatments and in some cases by raising the progeny and establishing them in replicated tests. This required the development of controlled pollination techniques which involved the isolation of the female organs, testing the effectiveness of the isolation and finding ways of handling the pollen.

In addition, various aspects of fruit maturity, causes of capsule loss, seed handling, seedling development and related matters had to be studied.

Chapter 2

REVIEW OF LITERATURE

Reference is made below to the work done with a considerable variety of genera, in addition to *Eucalyptus*.

2.1 Habitat of *E. grandis*

Hall *et al.* (1963) show *E. grandis* to occur along parts of the coastal belt of Queensland and of New South Wales, typically on alluvial flats and the lower slopes of the main valley systems. The climate is described as sub-tropical with a summer rainfall of 1 016 mm to 1 778 mm and no extremes of temperature other than light frosts in the valleys. The Forestry and Timber Bureau (1957) gives the range of altitude to be from sea level to 305 m in New South Wales and up to 762 m in Queensland. The species is typically found in pure, or almost pure stands in mixture with *E. pilularis* or *Tristania conferta*.

It occurs between latitudes 17 and 32 degrees South, compared with the closely related *E. saligna* which occupies similar coastal areas, but between latitudes 28 and 35 degrees South. There is thus an overlapping in the distribution, but with the latter species generally occupying a more southerly situation.

2.2 The inflorescence and flower in the genus

According to Jacobs (1955), the leaves arise from the growing tips in opposite or sub-opposite pairs, successive pairs being at right angles to each other. Pryor (1954) states that the opposite decussate branching pattern may be expressed in one form to give a leaf bearing system and in another form to give a flower bearing system. The various ways in which the inflorescence has been regarded are summarised and reference is made to Rickett's (1944) review of the classification of the inflorescence of Dicotyledons. From observations of the number of flowers and bracts in the bud and their symmetrical arrangement, the flower cluster is taken to be dichasial in nature, although it has the appearance of an umbel and that term is retained.

Carr & Carr (1959a) state that the unit inflorescence may consist of either three or more flowers, or of a single flower and is, with few exceptions, axillary. Its dis-

position along the annual shoot varies from basitonic to acrotonic in different species.

According to these authors, the subtending leaf is the bract of the central flower. It is sometimes abnormal in that it lacks a marginal meristem and withers at an early stage, in which case the term "prophyll" is applied to this organ. The other bracts form an involucre round the inflorescence and a relationship is traced between the numbers of bracts and of flowers in the umbel. For example they show that in the seven-flowered condition there may be 14 bracts, or numbers variously reduced to ten or six owing to the failure, especially of the bracts of series three, either to form or to persist. The stage at which the bracts are shed also varies as does the extent to which they are fused or free.

The floral axis in the genus is a cup like receptacle which encloses the ovary and which bears on its rim the stamens and one or two cap like structures, the opercula, representing the perianth. Pryor & Knox (1971) state that in those species in which the numbers of separate sepals and sutures between the petals can be seen, the floral whorls are tetramerous. The ovary commonly has four to six locules and is described by Carr & Carr (1962a) as being inferior or semi inferior.

Development of the flower, sporogenesis and embryology in *E. camaldulensis* are reported on by Zucconi (1959) and development of the gametophytes in *E. melliodora* and *E. stellulata* by Davis (1968 and 1969). Davis, in describing flower behaviour, also quotes Polunina (1959) who sets out events according to the number of days from anthesis in *E. macarthuri, E. cinerea* and *E. occidentalis.* In connection with breeding for cold resistance in Greece, Panetsos (1969) gives dates of flowering and seed production in *E. camaldulensis.*

2.3 Pollination

The authors who refer to pollination and related subjects in the eucalypts include Krug & Alves (1949), Pryor (1951), Penfold & Willis (1961), Venkatesh & Kedharnath (1965), and Mergen *et al.* (1966). The general impression conveyed for the genus as a whole is that flowers are mainly insect pollinated, that they are protandrous and that by the time an exudation occurs on the stigma, indicative of a receptive condition, much of the pollen has been shed. It is concluded from this and from the arrangement of the floral parts, that cross-pollination is favoured in any one flower, although it is of course recognised that some selfing is liable to take place.

2.4 Pollinating agents

According to Pryor & Boden (1962), in Australia, eucalypt flowers are visited by many species of insects in different groups. These authors refer to the effectiveness of species of blowflies (*Calliphora*) as pollinators while in another case, stress is laid on the usefulness of bees for seed production in *E. diversicolor* (Forestry Department Western Australia, 1966/67). There are also cases of birds taking nectar and pollen (Christensen, 1971b).

Krug & Alves (1949) refer to bees as important pollinators in Brazil and in South Africa, for beekeepers, May (1969) lists "saligna" as one of the nectar and pollen producers among the eucalypts.

A statement by Grant (1950) may also be relevant to the pollination of eucalypts. This is to the effect that some of the Lepidoptera are completely adapted for pollination while others, also some members of the Coleoptera and Diptera, are partially adapted.

Regarding the habits of pollinators, Grant also refers to the ability of bees to distinguish between certain colours and odours, as well as flower form. He mentions the instinctive foraging of bees over a limited area of a few square yards, or over a wider range depending on the relationship between the bee and the flower populations. This observation about limited foraging is supported by statements by May (1969), one of which is to the effect that out of 66 bees, only 21 were seen to visit more than one tree.

In connection with the above mentioned pollination by birds, the significant difference between birds and insects is pointed out to be the wider range of movement, also foraging under relatively inclement weather conditions.

2.5 Pollen viability

Pollen morphology in some of the Myrtaceae including *E. grandis,* is described by Pike (1956).

In connection with the need to store pollen for breeding purposes, Gabrielli *et al.* (1965) report that in certain eucalypts, the best method of storing pollen for maintaining viability is in a desiccator at 4 degrees C. Boden (1958) recommends deep freeze storage at −16 degrees C. He also refers to the improvement in germination obtained as a result of desiccation prior to testing. Pollen was viable after seven

months in the deep freeze compared with no germination after storage for one month at room temperature.

2.6 Receptivity

Assessments of receptivity in tree species include those made in *Fagus* by Nielsen & de Muckadell (1954), in *Castanea* by Nienstaedt (1956), in *Pseudotsuga menziesii* by Barner & Christiansen (1962), in *Araucaria* by Nikles (1965), in *Acer saccharum* by Gabriel (1966) and in *Liquidambar styraciflua* by Wilcox (1967). The information collected is of practical value in carrying out controlled pollination operations, especially where results include a record of the appearance of the stigmas at the stage of maximum receptivity.

There were two main bases of assessment, seed yield and germination of pollen on the stigma.

Barner and Christiansen did a series of pollinations on three clones, every one to two days for a period of three weeks, the results being based on seed weights and percentage germination from pollinations at stated dates.

Gabriel used flower buds placed in water in a greenhouse, which he pollinated with stored pollen 7,5 hours after bud burst and on a further 13 occasions at intervals of four to ten hours, up to the 102nd hour. Stigmas were taken for counting the number of germinating pollen grains after set periods of four hours. For this purpose paraffin embedded sections were prepared, stained with safranin and fast green.

2.7 Controlled pollination

Descriptions of the necessary preliminary flowering studies and of procedures based on these studies for controlled pollination are available for a considerable variety of tree species, including *Fagus* (Nielsen & de Muckadell, 1954 and Blinkenberg *et al.*, 1958), *Pseudotsuga menziesii* (Orr-Ewing, 1956), *Acer saccharum* (Gabriel, 1961), *Tectona grandis* (Bryndum & Hedegart, 1969) and *Pinus sylvestris* (Brown, 1971).

As regards eucalypts, some information is given by Krug & Alves (1949), Pryor (1951 and 1957), Shelbourne & Danks (1963), and Venkatesh & Kedharnath (1965). From a search of the literature, it appears that relatively little work has been done

on the effectiveness of the controlled pollination operation in eliminating contamination, although Pryor (1951) mentions the possibility of pollen passing past the fabric of muslin bags. Eldridge (1970) confirms this experimentally for this type of bag and reports that contamination is avoided by the use of unwoven terylene or cellulose sausage casing. This author, as well as Pryor & Boden (1962) used pollen-free blow flies within the bags as pollen vectors.

2.8 The fruit and seed

Carr & Carr (1962a) refer to the fruit in the genus as a loculicidal capsule surrounded by the enlarged hypanthium.

Pryor (1951) states that *Eucalyptus* fruit is ripe ten to twelve months after pollination, also that the seed may be viable a little earlier, but that the fruit may be difficult to open. Later (1956), he states that capsules ripen in many species in eight or nine months.

Penfold & Willis (1961) state that as far as is known, parthenocarpy does not occur in the genus, while according to Pryor (1951), the unfertilised fruit does not as a rule develop.

Christensen (1971a) describes how seed shed occurs in *E. diversicolor* as a result of drying, followed by expansion of the locules and the opening of the valves at the top of the fruit. The septa also split away from the placental column and the walls of the locules split at their weakest point. This may occur after fire, or more naturally after the formation of abscission layers at the base of the peduncles and pedicels.

Cremer (1965) describes the mechanism of seed shed for several species, (excluding *E. grandis*), in which various tissues shrink and cause widening of the locules and opening of the valves.

The seed is always mixed with particles commonly known as chaff and from anatomical examination, Gauba & Pryor (1958) distinguish, in *E. microcorys,* between two types of chaff particles. There are those which are thought to be from fertile but unfertilised ovules and those from infertile ovules. The former are similar to fertilised ovules in having a ventral hilum, but the latter have a basal hilum and poor tissue differentiation.

Larsen (1965) subdivides 300 species into four classes according to the extent to which it is possible to differentiate between seed and chaff by size and colour. He

classifies the seed of *E. grandis* as being different from the chaff in both of these features. He also states that the seed of eucalypts remains viable for many years.

2.9 Seed handling and testing

A recent compilation of conditions for testing the viability of the seeds of 350 *Eucalyptus* species is given by Scott (1972).

The main conditions laid down by the International Seed Testing Association (1966) for germination tests of small seeded *Eucalyptus* species are, seed on top of germinating medium (paper) at 20 degrees C. and in the presence of light. For reporting germination, since it is not feasible to count out seed lots of 100, units of 0,25 g are weighed out. The last count of seedlings is made after 21 days.

In testing the light requirements of 40 eucalypt species, Clifford (1953) obtained apparently conflicting results until the maturity of the seed had been taken into account. Immature seed of some species did not germinate satisfactorily until continuous light of about 200 ft-candles was given. On the other hand, mature seed of 38 out of 40 species tested did not require any light.

When investigating dormancy, Larsen (1965) used similar conditions to those laid down by the International Seed Testing Association, but instead of closing a test after the 21st day, he continued observations until no germination occurred for a week. No pre-treatment for the breaking of dormancy is indicated by the I.S.T.A., but Larsen classifies 300 species into those which have seeds which are dormant, partially dormant, occasionally dormant, or non dormant. He places *E. grandis* in the occasionally dormant class and recommends four weeks moist storage at one to four degrees C when it is required to break down this condition.

Banks (1968) reports that after scarification of the seed coat, the percentage germination increased and dormancy was broken in most of the species tested while Bachelard (1967) states that mechanical resistance of the seed coat is the primary cause of dormancy in two species examined.

Regarding a definition as to what constitutes a germinated seed, Larsen regards seed as viable when the radicle protrudes 1 mm or more. On the other hand, for seed testing, the I.S.T.A. definition emphasises the need to allow the seedling to develop sufficiently for it to be possible to assess whether it will develop into a normal plant.

2.10 Breeding systems

As an introduction to a consideration of the features which control inbreeding and outbreeding in plants generally, reference is made to authors such as Bateman (1952), Haskell (1953b), Fryxell (1957), Williams (1964) and Hagman (1967).

The relative amounts of self- or cross-pollination which occur in nature may be controlled by certain floral adaptations. In some species self-pollination is promoted in varying degrees by cleistogamy, or by the arrangement and development of the stamens in relation to the stigma. But other adaptations have the opposite effect, as in the case of flowers with exserted styles, or when there is polymorphism in style length and anther position.

Other systems which encourage cross-pollination are dioecy which precludes selfing, while monoecy tends to have the same effect, depending on the extent of spatial separation of the sexes and the separation in time of their maturing. Such dichogamy may also favour cross-pollination in hermaphrodite flowers and may refer to individual flowers, or to the whole plant.

Once pollination has occurred, fertilisation may be influenced by incompatibility, which is defined by Snyder (1959) as a failure or partial failure in some process leading to fertilisation; this may affect pollen germination, tube growth, or fertilising capability. A part may also be played by selective fertilisation, which involves the concept of competition between pollens.

Subsequent to fertilisation, infertility may result from embryo failure, or inability to survive may set in at a later stage. In species in which outbreeding has a selective advantage, this must act in favour of outcrossing by reducing the survivals in any ill adapted selfed progeny.

2.11 Breeding systems in trees

Perennials are reputed to be predominantly outbreeding and the indications are that self-fertilisation is rare in *Castanea* (Nienstaedt, 1956), in *Abies* (Klaehn & Winieski, 1962), in *Fagus, Betula* and *Alnus* (Hagman, 1967) and in *Platanus occidentalis* (Beland & Jones, 1967). Nevertheless many tree species have been found to include individuals which are self-compatible in varying degrees and to some extent self-fertile. According to the summary given by Hagman (1967) this applies to the genera *Pinus, Pseudotsuga, Picea* and *Larix,* and other genera listed by Bingham & Squillace (1955) include *Tsuga, Acacia, Acer* and *Quercus.*

One feature of reports on the above is the wide range of self-compatibility or self-fertility between individuals as noted in *Caragana arborescens, Pinus elliottii, Liriodendron tulipifera* and *Acer saccharum* (Cram, 1955; Kraus & Squillace, 1964; Taft, 1966 and Gabriel, 1967).

Variation between seasons is referred to by Nielsen & de Muckadell (1954) in *Fagus sylvatica* and by Bingham & Squillace (1955) in *Pinus monticola,* while Hagman (1967) mentions possible variation in *Alnus* in different geographical areas.

2.12 Notes on eucalypts

Krug & Alves (1949) obtained only negligible amounts of viable seed from seven species which were selfed, including *E. grandis,* and none of the seedlings survived. These authors are quoted by Fryxell (1957) when recording the same species as being self-incompatible. Wright (1962) also lists six species, including *E. grandis,* which are self-sterile.

On the other hand Pryor (1957) gives a list of nine species, not including *E. grandis,* which are fully self-fertile, only one tree of one species (*E. bicostata*) being fully self-incompatible. Also, out of 16 *E. regnans* trees tested, Eldridge (1970) reports that 15 were self-fertile to some extent.

2.13 Relative yield

This term is applied to the relative yield of seed resulting from self- compared with cross-pollination. This was investigated in *Pinus monticola* by Bingham & Squillace (1955), who did controlled self- and cross-pollinations, the latter usually involving more than one cross pollen in mixture. Taking the yield after cross-pollination as 100% "cross-ability", the relative yield (or "self-ability"), was calculated from:–

$$\frac{\text{Number of sound seeds per cone, self}}{\text{ditto cross}} \times \frac{\text{\% germinability, self}}{\text{ditto cross}}$$

Using this formula, the results for different trees varied from 5% to 124% with a mean of 51%.

Other workers on this and related subjects include Beland & Jones (1967) with *Platanus occidentalis* and Sorensen (1971) with *Pseudotsuga.* In the case of the latter, an average relative yield of 11,3% is reported while in *Platanus,* mean germination percent after selfing is given as between 0,2 and 1,2%.

2.14 Inbreeding effects

Pryor (1961) states that marked depression of growth can occur following inbreeding in eucalypts, but after limited tests with *E. grandis,* Burgess (1973) reports no difference between outcrossed and selfed progeny in seeds per capsule, survival or growth. Apart from such statements, by far the majority of reports on this subject are concerned with a wide variety of other genera, in which inbreeding commonly results in reduced seed yield, decreased vigour and irregularities in form and pigmentation.

Fowler (1965a) records that selfed seedlings of *Pinus resinosa* show little or no inbreeding depression, but Squillace & Kraus (1963) and Kraus & Squillace (1964) report that this is unusual among pines. Other workers have reported inbreeding depression in other genera, including some of those mentioned on pages 9 and 10.

Williams (1964) stresses the significance of vigour as a guide to the breeding system; species which are naturally outcrossing are invariably subject to reduced vigour after controlled selfing.

Pawsey (1964) made observations of height growth in inbred progeny of *Pinus radiata* until they were eight years old and Snyder (1972) in *P. elliottii* until they were five years old. Other workers made comparisons of self and cross progeny in the nursery only. Barnes (1964) reports that in *Pinus monticola,* the heights of selfed offspring from completely self-fertile trees were less depressed than those from trees which were only partly self-fertile. In the case of *Cryptomeria japonica,* heights after selfing were more variable than after crossing (Ohba & Murai, 1969).

Irregularities in form may include an effect on stem straightness, but this is difficult to assess satisfactorily. The most effective methods are apparently those based on photography, e.g. Shelbourne (1967) and Vidakovic & Ahsan (1970). Less involved methods include those by Goddard & Strickland (1964), Keiding & Olsen (1965), Bannister (1966), Shelbourne & Stonecypher (1971) and Slee (1971).

Franklin (1968) gives detailed tabulated summaries of abnormalities and inbreeding effects in conifers and the same author (1969) describes a large number of mutant forms in *Pinus taeda,* mostly connected with pigmentation. In discussing albinism and other types of chlorophyll deficiency, Squillace & Kraus (1963) list several genera in which this has been noted, including *Pinus* and *Picea* species, *Castanea, Populus, Acer* and others. Various degrees of deficiency may be encountered affecting the whole or

only part of the plant and developing at the seedling stage or later. Deviant seedlings may develop normal pigmentation later, or the reverse process may occur.

Snyder *et al.* (1966) describe abnormalities in colour of the cotyledons of *Pinus elliottii*. They classify these into three groups, mutant plants of one colour, mutant plants of two or more colours and plants showing changes in colour. Within each of these groups, terms are applied according to the various colours and the changes which occur in them.

2.15 Natural selfing

Inferences may be drawn about the breeding system of a plant from its flower morphology and related floral characters (p 9). Fryxell (1957) refers to this subject, but also to the limited usefulness and applicability of such observations. In regard to entomophilous species, he points out that the types of insects present, their activity and their number in relation to the numbers of flowers and weather conditions may all have an effect on the breeding system. In addition, as already indicated, seasonal differences and environmental factors may also affect the issue.

Fryxell describes procedures for assessing the percent natural crossing using gene markers which should, *inter alia,* preferably be expressed phenotypically at an early stage and should not be of such a nature as to have any effect on pollination. But concerning this method Franklin (1971b), expresses the opinion that insufficient emphasis has been given to the effect of embryo mortality; surviving seedlings may not be representative of the actual number of fertilisations.

Franklin (1971a) estimated percent natural self-fertilisation in *Pinus taeda* on the basis of percent filled seed in cross- (C), wind- (W) and self- (S) pollinated progeny, using the formula (C–W/C–S) 100.

Taft (1966) observed characters such as percent filled samaras, survival and height in *Liriodendron tulipifera*. Progeny from open-pollination were generally intermediate between the self and cross, and it was concluded, without any actual calculation of the amount of selfing being made, that the open-pollinated progeny consisted of a mixture from both self- and cross-fertilisation.

Using a genetic marker in the form of seedlings with leaves of a lighter blue-green colour than normal, ratios not differing significantly from three normal to one deviant were obtained by Philp & Sherry (1946) after selfing in *Acacia decurrens*. On the basis of each light coloured seedling representing four selfs (or using the

formula A-3a/A+a, Fryxell, 1957), the natural crossing was estimated at between 81% and 100%. The results were regarded as providing minimum estimates since no account was taken of the numbers of carriers in the stand, as was done by Squillace & Kraus (1963). Using albino markers, on the basis of results from one tree, the latter assumed a ratio of 3:1 in all self-pollinated progeny of *Pinus elliottii* and found that selfing varied from 0% to 27% in eleven trees.

As in the above cases, various deviant types are often stated to be due to a single recessive gene, although ratios differing widely from 3:1 are sometimes obtained. Sorensen (1967) refers to factors which may affect this issue, including difficulty in identification (possibly connected with sensitivity to the effect of environment), sampling error in small selfed families, and abortion of weaker deviant embryos.

Fowler (1965b), using *Pinus resinosa* showing various degrees of chlorosis, obtained a ratio of 4,9:1 after selfing and used this ratio in estimating natural selfing, assuming that all deviant seedlings in wind-pollinated progeny were derived from selfing. Similar work was done with *P. banksiana* (Fowler, 1965c).

As regards eucalypts, Krug & Alves (1949) mention an estimated 77,2% natural crossing in *E. alba*. Albino seedlings were used which, being easily classified and occurring at an early stage, had two desirable attributes for a marker.

Using "curled" seedlings as markers in *E. regnans,* Eldridge (1970) estimated 28% selfing in the lower crown and 8% in the upper crown.

Other reports for the genus on this subject are of a more general nature. Florence (1964) refers to evidence that, in connection with variation in time and intensity of flowering, fertilisation in one *E. pilularis* tree resulted almost exclusively from self-pollination. However, Pryor (1957) maintains that "there must be rather a high amount of intra specific out crossing, whatever the precise mechanism, to maintain the genetic diversity which *Eucalyptus* species usually show". He also states (1961) that while selfing occurs, it does so less readily than out crossing, while Christensen (1971b) quotes other authors who report that *E. ficifolia* is entirely cross-pollinated in nature.

According to Pryor & Johnson (1971) apomyxis is unknown in the genus.

2.16 Barriers to selfing

Of the possible barriers to selfing referred to on page 9, aspects investigated in tree species include incompatibility, infertility and selective fertilisation.

Bingham & Squillace (1955) define self-compatibility as the ability to produce pollen that can fertilise ovules of the same tree and self-fertility as the ability to produce viable self-fertilised seed. Using the expression shown on page 10, self-compatibility was judged according to the number of sound seeds per cone after selfing compared with crossing, and self-fertility from the relative figures for percent germination. For *Pinus monticola* the yield of sound selfed seed and percent germination averaged about 50% and 90% respectively of that from crossing and the sample was classed as moderately self-compatible and highly self-fertile.

Hagman (1963) reports a retardation in growth rate of self pollen tubes in the style of two species of *Betula.* He also states that the incompatibility reaction is slowed down by low temperature and that this may also occur when pollination is done at a late stage in female flower development. Williams (1964) refers to the same effect in flower buds and end of season flowering.

In most tree species reported on, reduced seed yield after selfing, rather than being due to incompatibility, is most commonly associated with post-fertilisation abortion of ovules, as reported for *Liquidambar styraciflua* and *Acer saccharum* (Schmitt & Perry, 1964; Gabriel, 1967). Similar results are given for *Pinus, Picea* and *Larix* by Hagman (1967) while Mergen *et al.* (1965) found that there was no barrier to self-pollination or self-fertilisation in *Picea glauca,* but that breakdown began early in embryo development. However, Hagman & Mikkola (1963) found that in the case of *Pinus peuce,* early embryo development after selfing was comparable to that after crossing.

Selective fertilisation, which is another possible barrier to selfing, is referred to by Bingham & Squillace (1955) as "the relative efficacy of pollen from a given plant in fertilizing ovules within its own flowers when in competition with pollens from other plants of the same species". Squillace & Bingham (1958) considered its overall effect, covering "all types of discrimination in reproduction" — "occurring in any stage of the reproductive cycle".

The latter authors as well as Barnes *et al.* (1962) made use of the reduced vigour found in selfed compared with crossed progenies of *Pinus monticola* to assess the proportion of these likely to be present after mixed-pollination; the criteria used were the relative values in the three treatments for germination time, epicotyl length and height. If significant differences were found in the above features, also in seed yield and numbers of cotyledons, and if the progeny from mixed-pollination was near-

er to the self or to the cross, it was concluded that selective fertilisation was likely to have occurred.

An albino marker was also used as a check on these results and it was concluded that selective fertilisation had occurred in favour of cross pollen, at least in the less self-fertile trees.

Fowler (1965b) examined several possible factors which might act as barriers in *Pinus resinosa* and found that the presence of functionally male trees and the relative positions of the male and female "flowers" were important. Natural selfing was higher in the lower part of the crown and the latter result was also obtained for *P. banksiana,* selfing being 13% in the upper crown and 26% in the lower crown (Fowler, 1965c). Corresponding figures obtained by Franklin (1971a) for *P. taeda* are 7% and 34%.

Fowler (1965b) also tested selective fertilisation, using normal to deviant ratios after separate self- and cross-pollination to state expected ratios in a theoretical mixture of equal amounts of these two pollens. The latter did not differ significantly from results observed after applying an actual mixture of the two pollens and it was concluded that there was no evidence that selective fertilisation had occurred.

Chapter 3

THE PRESENT TAXONOMIC POSITION OF *E. GRANDIS*

3.1 Taxonomic evidence

Features of the anther, first used by Bentham (1867), have continued in use for many years for subdividing the genus into major taxonomic groups. Recently however, the antheral classification has been subject to much criticism, mainly because of the over-emphasis on a single floral character (Carr & Carr, 1959b).

Evidence of likely taxonomic significance is now being considered from many other sources, including the distribution of the ovules and ovulodes (Carr & Carr, 1962b), the oil glands and ducts (Carr *et al.*, 1970) as well as ovule and seed structure and anatomy of the integuments (Gauba & Pryor, 1958, 1959 and 1961). The work on seedling morphology by Brooker (1970) is also worthy of mention.

In regard to features of the perianth, Pryor & Knox (1971) describe in detail their mode of origin and their subsequent development in a wide range of species representing the genus. Four types or "pathways" of development are recognised. In one, petal initiation is suppressed and there is only a calycine operculum, but in species with both perianth whorls represented, there are three types according to the timing of initiation of the petal primordia in relation to that of the sepals and according to the form of the sepals in the young flower bud. Four different types of developed flower bud are also described.

These perianth types, as well as characters of the inflorescence, anthers, ovules, cotyledons and certain other vegetative features are referred to by Johnson (1972) for each of eight proposed subgenera. The main types of inflorescence are terminal, or end in a vegetative bud which either aborts or continues growth. The main anther types are illustrated according to shape, type of dehiscence and whether versatile or adnate. The ovules are anatropous or hemitropous and reference is made to the possible value of further investigations of placentation and features of the ovary and style. The cotyledons may be entire, emarginate, or Y shaped. Moggi (1961) also includes the reniform shape in this tentative key for identification of seedlings according to cotyledon and hypocotyl characters.

In addition to evidence from morphology, Pryor & Johnson (1971) also refer to that from chemistry and genetics.

3.2 Systems of classification

In his key, in which over 500 species are described and classified and which provides cross references to the work of Maiden (1903–1931), Blakely (1955) divides the genus into sections and lower ranks of taxa. For example, *E. grandis* is placed in the section *Macrantherae* (p. 18) and the subsection *Longiores* on anther characters. By further subdivision he places the species in the series *Transversae* and in the subseries *Leptocarpae* for which features of the inflorescence, flower buds, opercula, fruits and certain vegetative characters are described.

Carr & Carr (1962a) recognise two groups which they regard as distinct at the generic level, namely *Eucalyptus* and *Symphyomyrtus,* although this sub-division of the genus does not appear to be generally accepted.

Pryor (1956) states that field evidence and controlled experiment have shown that the genus is divided into four or five major systematic groups which are largely or completely reproductively isolated from one another.

More recently Pryor & Johnson (1971) evolved a revised system which takes into account the evidence available at the time. They devised a system of 1- to 6-letter coded designations representing infrageneric subdivisions. The first letter of each subgenus is used as the initial letter of the code, subsequent letters of which represent further subdivisions into five ranks of taxa, including subspecies, the letters often being chosen to give words which can be pronounced.

The coded designations serve to show relationships between taxa and, as stated by Pederick (1972), the system allows for revision in a way not possible with the numerical ordering of species used by Blakely.

The authors propose that the eucalypts (and *Angophora*) should be considered as consisting of eight taxa, for the time being ranked as subgenera; these correspond, in general, to fertility groups.

Of these eight subgenera, only three are commonly represented in plantations in South Africa, so that our range of practical experience of the genus is very limited. There are the subgenera *Corymbia* which includes *E. maculata,* and *Idiogenes* with the single species *E. cloëziana.* The third subgenus is *Symphyomyrtus,* which includes *E. grandis* and many other species.

3.3 The subgenus *Symphyomyrtus*

This is a large group in which, according to Johnson (1972), the inflorescence is of the terminal type or ends in an active vegetative bud, the ovules are hemitropous, and the cotyledons are either emarginate or Y shaped.

Since the subgenus is equivalent to Blakely's sections *Porantheroideae-Terminales* and most *Macrantherae* (Pryor & Knox, 1971), there is a great diversity of anther types. As illustrated by Johnson (1972), these may vary from versatile to adnate. Loculi may be parallel or somewhat divergent and some may be confluent at the top. Dehiscence may be in long separate slits, or in pore-like openings which may be terminal.

There is a petaline operculum with free caducous sepals, or sepaline as well as petaline opercula which are shed separately, or tend to be closely appressed to form an apparent single operculum.

According to Pryor & Johnson (1971) there are 11 sections within *Symphyomyrtus* those of most interest in South Africa being *Exsertaria* including the species *E. alba* and *E. camaldulensis, Maidenaria* (*E. globulus* and others), *Adnataria* (*E. paniculata* and others), *Sebaria* (*E. microcorys*) and *Transversaria,* which includes *E. grandis.*

3.4 The section *Transversaria*

The coded designations by Pryor and Johnson are illustrated below by setting out the position of *E. grandis* with reference to the more closely related species within the section *Transversaria:*

Subgenus Symphyomyrtus		Code *S*
Section Transversaria		*SE*
Series Diversicolores		*SEB*
Subseries		
Species	E. diversicolor	SEB:A
Series Salignae		*SEC*
Subseries Saligninae		*SECA*
Species	E. deanei	SECAA
	E. grandis	SECAB
Super species saligna	E. saligna	SECAC
	E. botryoides	SECAD
	E. robusta	SECAF

Information about the more recently investigated features such as initiation of perianth primordia is not available for *E. grandis,* but the related species *E. botryoides* is recorded by Pryor & Knox (1971) as having petals initiated soon after the sepals.

3.5 *E. saligna* and *E. grandis*

Some reference must be made to differences between *E. saligna* and *E. grandis,* in view of the confusion about these two species in this country. As stated by Marsh (1953), this arises from the fact that it was not until 1918 that Maiden recognised the latter as a separate species. Marsh records the differences between the two according to those publications which recognise *E. grandis* as a separate variety or species, namely Baker & Smith (1920) and Blakely (1934).

Perhaps the most distinctive feature mentioned by Marsh is the valves of the fruit. These are clearly seen in various published illustrations, e.g. the Forestry and Timber Bureau, 1954, 1957 and 1968. According to Marsh, in *E. grandis* there are four to six valves, usually five, compared with three to five (usually three or four) in *E. saligna.* In *E. saligna* the valves are exsert, straight or spreading, fragile, pointed and easily broken, whereas in *E. grandis* they are exsert, incurved and blunt at the tips.

Pryor (1961) refers to the fact that *E. saligna* has lignotubers (Chattaway, 1958), while *E. grandis* has none.

Locally-observed differences of practical importance are that *E. grandis* flowers much earlier in the season and it is much easier to de-bark than *E. saligna.*

Chapter 4

MATERIALS AND METHODS

4.1 Materials

The only immediate sources of breeding materials are the existing plantations in South Africa which are derived from importations of seed made during the second half of the 19th century (van Laar, 1961). The precise origins of this seed in Australia are largely unknown, but it was probably labelled *E. saligna,* which is the name popularly used, although *E. grandis* is more common (Streets, 1962).

These plantations, in which selections are being made, are scattered over a wide range of sites in the Northern and Eastern Transvaal and in Zululand. Of the twelve clones which had been selected and accepted for breeding purposes by 1966, the first five to come into flower form the main subjects for the initial investigations. These are referred to as the "main clones" or, specifically, G6, G10, G15, G17 and G19. Other clones were incorporated later as they came into production.

Controlled pollinations were done mainly in the tree bank (p. 1), but with extra work in the seed orchard as required. The tree bank is near Tzaneen in the Northern Transvaal at an altitude of 760 m. For the seven seasons from 1966 to 1972 it had a mean annual rainfall of 929 mm on 77 rain days. Frosts are occasional and usually light. There is no clearly defined winter dormancy, but simply a decrease in the number of buds in active growth. The seed orchard is some seven miles to the west of the tree bank, at an altitude of from 1 200 m to 1 400 m where rainfall for the same period averaged 1 727 mm per annum on 102 rain days.

At both sites, a wide espacement of 8 x 8 m is adopted so that a branchy habit with maximum flowering space is encouraged. The tops of the ramets are trimmed back and growth is controlled in one way or another, to make access to flowers and fruits easy.

The seed orchard is surrounded by pine plantations and is well isolated from contamination from other sources of eucalypt pollen. The tree bank is not so well isolated, being separated by open spaces and other species such as *E. paniculata,* from the nearest other *E. grandis* plantation some 200 m away.

4.2 Methods

The methods described below are for controlled pollination and for seed handling. Methods in connection with flower morphology are included in Chapter 5.

Controlled pollination

Special pollination procedures, applied to specific tests, are dealt with under the tests concerned, but here, the procedures given are of more general applicability. An assessment is also included of the effectiveness of the operation as regards avoidance of contamination.

Although details of procedure for eucalypts would vary according to species, that for *E. grandis* was based on descriptions by Pryor (1951 and 1957) and others.

The first process, that of emasculation, was performed on flower buds which were at or approaching the "mature" stage, green buds and open flowers in the vicinity having been removed previously. This left up to a dozen buds at the required stage for inclusion in the bag.

A cut was made just below the staminal ring, using a cutting instrument of a type similar to that described by Meskimen (1965). The flower was, as well as possible, supported between the thumb and forefinger during this operation, so as to minimise twisting and pulling of the pedicel. The first cut usually removed the whole of the staminal ring, with stamens and operculum attached (Fig. 8 facing p. 29), but some anthers tended to remain behind at the base of the floral cup after emasculation, and these were removed with a pointed instrument before the bag was put in place.

After emasculation, a wire spiral was drawn over the branch so as to enclose it and to support a white cotton tube 27 cm long by some 10 cm wide (measured flat), commonly referred to as a bag. The spirals were kept in place and the ends of the bag closed by pipe cleaners, using cotton wool to limit abrasion.

For the purpose of a pollen supply the aim was, unless otherwise stated, to have flowers which had been open for one to two days and which had an accumulation of pollen on the anthers. For this purpose therefore, maturing flower buds were selected in advance and bagged.

Originally, the only method of applying pollen was by rubbing the stigmas with a few anthers, held by the filaments in a pair of tweezers, referred to as the

"by anther" method. Later, application "by brush" was introduced; this involved the removal of the anthers which were then dried in a desiccator with silica gel for an hour or more. The anthers were then crushed and mixed before application with a small paint brush. Match boxes were used as containers.

Pollination was most frequently done three to five days after emasculation, depending on requirements. Bags were unfastened and the pollen applied, then the bags were closed and left in place till the styles had fallen.

Before application of pollen by brush, it was considered desirable to mix the pollen in order to promote as far as possible, the application of comparable numbers of viable grains to each stigma. As to the application of comparable gross amounts of pollen, there was no control over this other than by rubbing on as much as could be made to adhere to each stigma.

Light waterproof covers were placed over bags covering newly pollinated flowers (also over bags placed for a pollen supply), as protection against possible rain. These were left in place for about four days after pollination of mature flower buds, from which pollen is easily lost (p. 74), or for one day in the case of fully receptive stigmas.

As regards the recording of controlled pollination operations, a standard field sheet was used for entering dates, bag and clone numbers, also the numbers of flowers pollinated and capsules reaped. Each bag was given a serial number for the year and this number (usually the first of the series) is quoted when reporting experimental results.

After pollination, flowers and capsules were examined periodically and if losses were seen to have taken place as a result of mechanical damage (p. 42), the numbers were reduced by deleting such flowers and capsules from the record. In the absence of any evidence of such mechanical damage, losses due to abscission following lack of fertilisation are likely (p. 43) and may occur more after some treatments than others. In this case, the capsules reaped would exclude some which had no seed, so that in tests based on numbers of seeds per capsule, results would be over estimated. Account was taken of this as far as possible in considering results.

As precautions against contamination by unwanted pollen, open flowers were removed from the site before emasculation and especially before pollination of re-

Table 1

Effectiveness of isolation during controlled pollination; numbers of seeds produced after emasculation and bagging

	Clone	Numbers of flws.[1]	caps.[2]	seeds
Bags not opened				
Flower buds immature	G3	100	99	0
	G15	39	33	0
	G17	23	0	0
Flower buds mature or mixed	G6	26	7	0
	G15	82	67	1
	G17	13	0	0
	G19	48	1	0
Bags opened 1 to 5 times				
Flower buds immature	G15	39	33	1
	G17	36	3	0
Flower buds mature or mixed	G6	36	21	0
	G10	24	0	0
	G15	88	52	0
	G17	119	1	3
	G19	164	1	0
	G35	8	6	0
		845	324	5

[1]flowers [2]capsules

ceptive stigmas. Stigmas were cleaned after emasculation by a small jet of water from a plastic bottle, or by blowing on them. Hands and instruments were kept clean and touching the stigmas with these or with the fabric of the bag was avoided.

Effectiveness of isolation

In order to test what contamination occurs during the controlled pollination process, flower buds were emasculated at various stages of maturity, followed by the placing of test bags, some of which were opened as if for pollination. This was done from 1966 to 1968 mainly in the tree bank and the numbers of flowers emasculated and bagged, capsules reaped and seeds germinated are shown in Table 1. The clone G3 was tested in 1965, before the writer's arrival at the station and it is assumed that the flowers were immature, as shown, though information on this point is not given in the field record.

The results emphasise the need for precautions against contamination and are recorded here as part of the controlled pollination method. A total of 324 capsules was produced from the 845 emasculated and bagged flowers shown in the table; the highest proportion of flower loss was in G10, G17 and G19 and was probably largely due to lack of fertilisation.

Five seeds germinated from the 324 capsules, three of these being from a single capsule of G17, the bag of which had been opened. Further information on this subject is obtained from Table 7 (p. 41) where, after emasculating and bagging flower buds approaching maturity, the bags not having been opened, five seeds were obtained from 324 capsules, giving and overall rate of contamination of ten seeds from 648 capsules.

Although no definite conclusions are drawn about the causes of contamination, opening of the bags and accidental touching of the stigma seem the most likely causes. Passing of pollen through the cotton bag is a further possibility, also pollination by traces of pollen left at the base of the floral cup after emasculation.

Seed handling

Under this heading, methods in connection with capsule maturity, seed extraction, the germinating conditions provided and the method of raising seedlings are described.

A maturation period for the capsules of at least five months was allowed at the tree bank and about seven months at the seed orchard (p. 40), after which seed extraction was done by air drying, or in an oven at 30 degrees C. Seeds at the base of the capsule tend to remain in place and to ensure their removal, it was found best to expose each locule by a transverse cut at the base of the capsule.

In view of the report by Larsen (1965) about limited dormancy in *E. grandis,* and the observation locally of only occasional delay in germination, no steps were taken in connection with the breaking of dormancy.

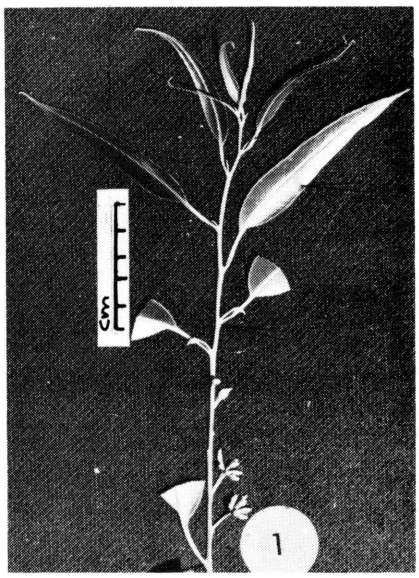
Initially, no equipment was available for germinating under controlled temperature conditions and porous germination dishes were used, standing in trays of water, indoors and with indirect sunlight. Subsequently a Jacobsen germinator was used in the great majority of tests, regulated at 25 degrees C.

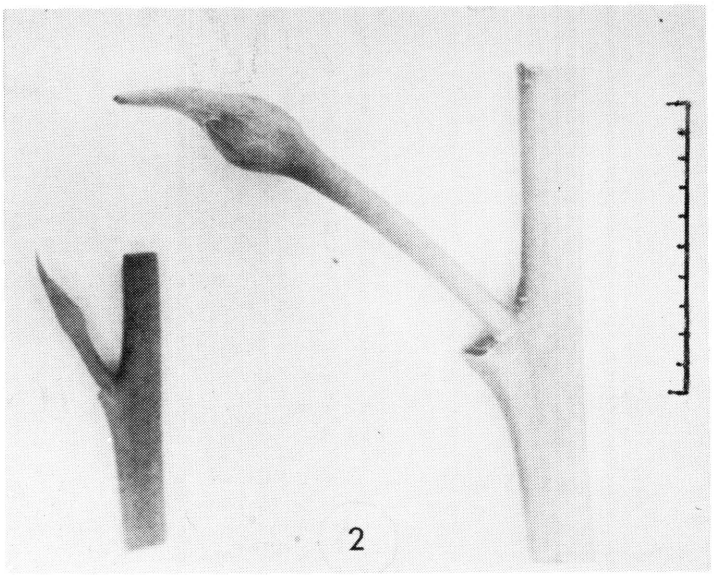
Where it was necessary to equalise the number of capsules available in each treatment, excess capsules were discarded randomly before placing the seed for germination. Except where otherwise stated, the contents of each capsule were then placed separately for germination.

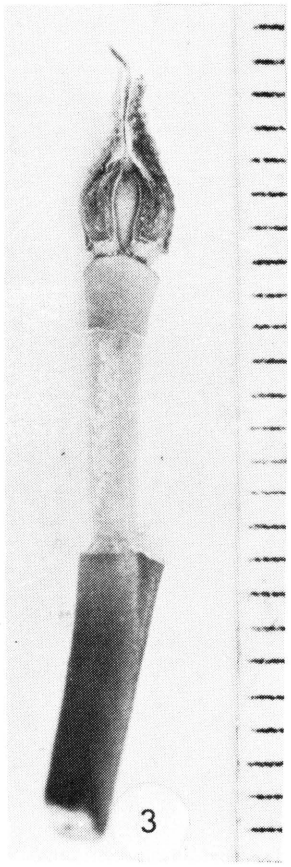
Germination was regarded as complete after 21 days. Seed was regarded as germinated when the radicle was clearly visible. Germinating seeds were transferred individually to polythene tubes 15 cm high by 10 cm wide (measured flat). These were filled to within some 3 cm of the top with thoroughly mixed and sieved nursery soil, and topped by a layer of finely sieved soil. Germinating seeds were drill sown and to avoid wash of newly planted seedlings, coarse sand was added as the hypocotyl lengthened.

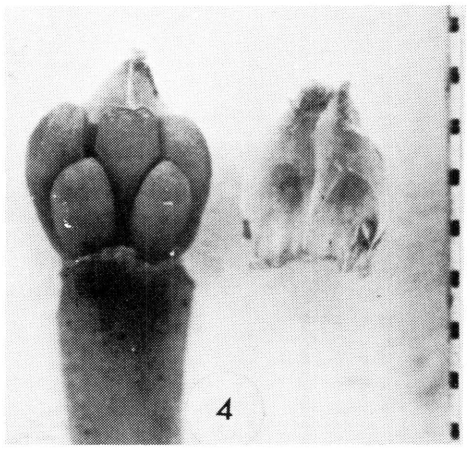
The pads of the germinator were inspected daily, or less frequently, depending on the type of test and on the stage which the germination had reached, i.e. whether or not it was at its peak. In all cases where tables have columns headed "number of seeds", this refers to the number of germinations in the germinator.

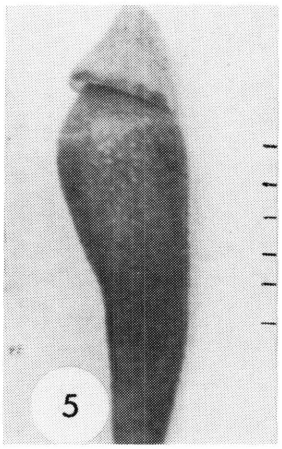
In certain cases where double germinations were found emerging from the soil after the seed had been transferred for planting (p. 50), these were counted as two seedlings. Where deaths occurred from insect attack or mechanical damage of the type recorded on page 47, the numbers concerned were disregarded in calculating survival.

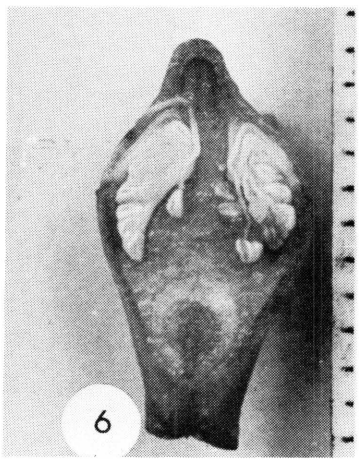












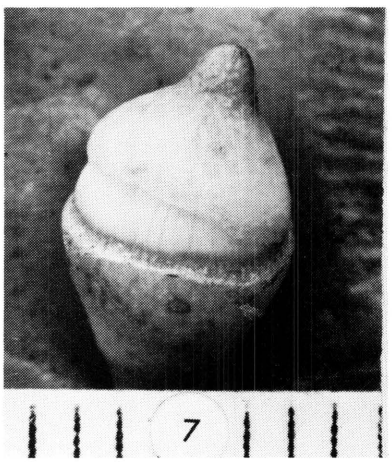


Fig. 1. Flowering shoot with 3 nodes of floral buds and distal nodes of vegetative buds. Scale in cm.

Figs. 2 and 3. Umbel buds before and during bract shed.

Fig. 4. Umbel bud with two outer bracts removed, also 2 abaxial inner bracts removed and placed on one side, leaving flower buds with 2 buds of series 3 on the adaxial side, still enclosed in 2 inner bracts.

Fig. 5. Flower bud with outer operculum being shed.

Fig. 6. Flower bud approaching anthesis cut longitudinally and showing inflexed stamens and inferior ovary.

Fig. 7. Inner operculum being shed.

Scales in mm.

Chapter 5

FLOWERING IN *E. GRANDIS*

5.1 The inflorescence and flower

The unit inflorescence is a single stalked dichasium commonly referred to as an umbel. The first few buds formed in the season are always vegetative and these are followed by floral buds. One to fourteen of these may be produced on any one branch in a season and the first and last may differ from each other both in time of formation and anthesis by several weeks. The formation of vegetative buds is then resumed (Fig. 1) and the umbels are thus mesotonically disposed on the season's flowering shoot.

According to Blakely (1955), the umbel is three- to ten-flowered and although some clones were noted at the tree bank with seven to 14 flowers in the 1973 season, during all other seasons the seven-flowered condition was typical. The umbel is initially enclosed in an involucre of bracts and, using the interpretation of Carr & Carr (1959a) the subtending leaf, which sometimes fails to develop fully and to persist, is the bract of the central flower (series 1). Its pedicel bears two bracteoles ("outer bracts") each subtending an axillary bud of series 2; the pedicels of these bear two bracteoles ("inner bracts") each with an axillary bud of series 3 (Figs. 3 and 4, and Fig. 13 facing p. 30). The pedicels of these may each also bear one or two small bracteoles and there are thus six to 14 enclosing bracts.

The receptacle of the flower is broadly conical and it tapers into a short pedicel, that of the central flower being slightly longer than the others. The calycine ring round the top of the receptacle carries the opercula and the stamens. There are two opercula (representing the calyx and corolla), which are shed independently of each other, and numerous stamens in a continuous ring, those inserted nearest the centre being shorter than those towards the periphery. In the bud, the stamens are inflexed, with the anthers arranged at the base of the floral cup. The top of the ovary extends just above the rim of the receptacle and bears a single style which is simple and subclavate with a single unlobed stigma which, in the bud, is closely enclosed in the tip of the inner operculum. The ovary is inferior (Fig. 6).

Table 2

Duration of umbel bud and of flower bud stages in the tree bank 1967/68

Clone	Nr. of umbels	Duration in days						Total
		Stage 1[1]		Stage 2[2]		Stage 3[3]		
		Mean	Range	Mean	Range	Mean	Range	
G6	3	48	43–51			27	26–30	
G10	4	42	40–43	41	33–47	31	28–42	114
G15	5	56	51–62	35	30–38	45	42–49	136
G17	5	48	45–50	40	35–49	30	21–34	118
G19	6	43	38–46	30	21–45			
G47	4	27	24–28	77	70–80	13	9–23	117
G50	6	32	21–42	48	37–61	32	21–39	112
G58	4	36	22–42	53	40–64	19	8–25	108

[1]Stage 1, umbel bud with the unit inflorescence enclosed in an involucre of bracts.

[2]Stage 2, flower bud with two opercula.

[3]Stage 3, flower bud with one operculum.

5.2 Flower phenology

Three stages in flower bud development were recognised and recorded at the tree bank in the 1967/68 flowering season. These are as shown in Table 2, the first being when the unit inflorescence is enclosed in its involucre of bracts, referred to as the "umbel bud" stage (Figs. 2 and 3). The second is when the bracts have been shed to reveal individual flowers with two opercula (Figs. 4 and 5) and the third when only the inner operculum is present (Figs. 6 and 7), referred to as "flower bud" stages.

It is possible to recognise a young umbel bud when it is about 3 mm long, by its slightly more swollen appearance compared with a vegetative bud. After about six weeks (three to nine weeks) the outer bracts start to separate from each other at their tips and/or lower down, and after the involucre has become detached along its line of insertion, it may be shed as a whole or as a number of variously fused or separate bracts.

The individual flowers with two opercula are then revealed and this stage may last for six weeks (three to eleven weeks), by which time the outer operculum is conical in shape and starts to become discoloured before being shed (Fig. 5).

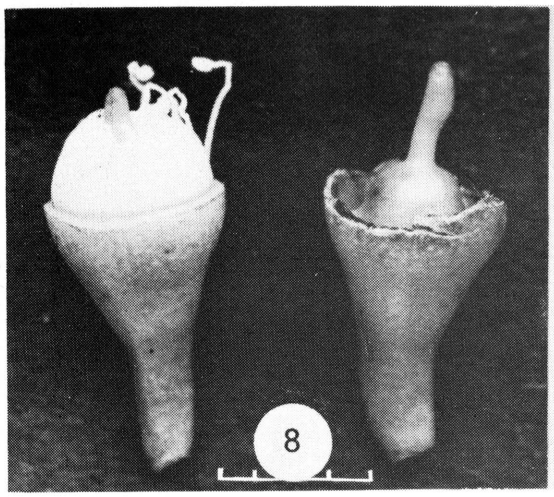
The inner conical to shortly rostrate operculum then remains in place for some four weeks (one to seven weeks). During this time it starts to turn yellow from the base upwards and shedding is imminent when this colour approaches the tip. This gradual change in colour gives a clear indication that the buds are nearly mature and at a suitable stage for emasculation.

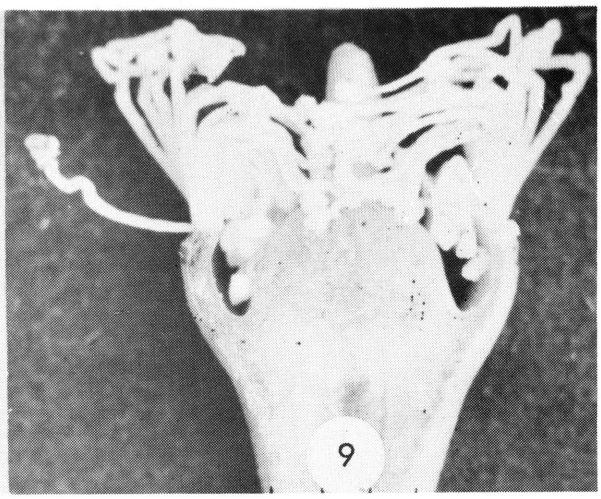
Apparently, increase in length of the style detaches the operculum from the calycine ring, then as the stamens begin to unfold, they function in lifting the operculum so that its tip no longer encloses the stigma (Fig. 7).

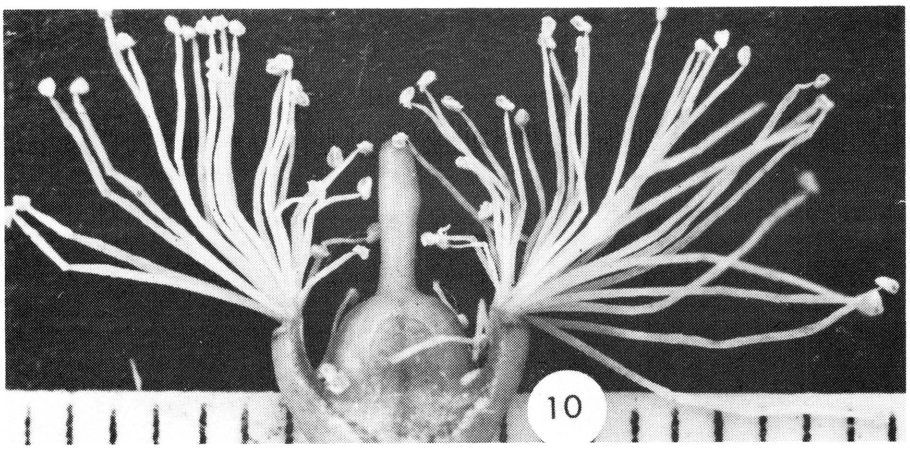
Shedding is usually completed within a few hours, but in G19, the inner opercula quite often remain *in situ* for an indefinite period.

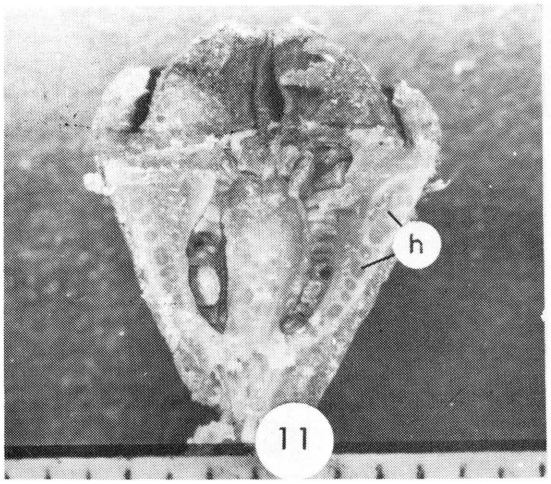
Another irregularity is the tendency towards simultaneous shedding of the two opercula, discernible from the figures for G47 and G58 in Table 2.

Although there is much variation between clones in the duration of any one of the stages, as may be seen from the table, this is less pronounced in respect of the total period from early recognition of the umbel bud until anthesis. This was about four months at the tree bank, but for 50 to 100 flowers of each of the five main









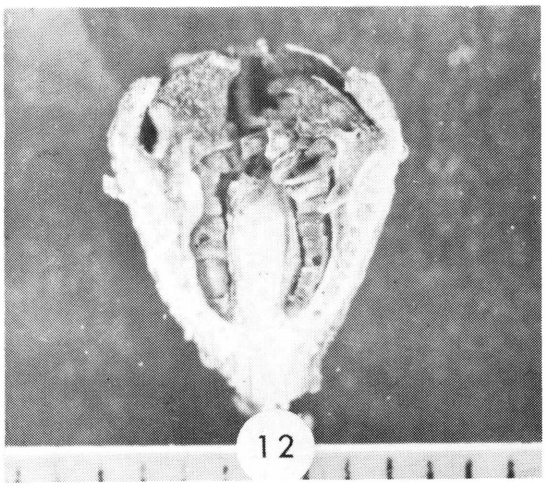


Fig. 8. On the left, a flower shortly after anthesis, on the right an emasculated flower bud.

Fig. 9. Flower cut longitudinally, a few hours after anthesis and shortly before the stigma becomes completely surrounded by anthers.

Fig. 10. Flower cut longitudinally showing unfolded stamens.

Fig. 11. Fresh fruit cut longitudinally, (h) hygroscopic tissue.

Fig. 12. Dried fruit with shrunken hygroscopic tissue, widened locules and valves open.

Scales in mm.

clones at the seed orchard in the 1969 season, the period was about five months in each case.

Changes taking place subsequent to anthesis are now dealt with, including the unfolding of the stamens, pollen shed and changes in appearance of the stigma, as observed for the five main clones in the tree bank.

When the inner operculum is first shed, the flower appears as in Figure 8, with the stamens inflexed, the anthers closely enclosed in the floral cup and the stigma protruding. Thereafter, the stamens usually start to unfold within a matter of minutes. The outer filaments first bend outward at the base, then the anthers are lifted from the inflexed position by straightening of the filaments.

A few hours after shedding of the inner operculum, the anthers pass very close to the stigma (Fig. 9) and unfolding is practically complete after one day, when the position shown in Figure 10 is reached, with the short inner stamens still quite close to the stigma. On the third day, the filaments start to become easy to detach from the staminal ring, withering begins on the fourth day and the process is usually complete after one week. In wet weather, nearly all the stamens are shed by the third day.

Pollen shed commences early; small amounts can be seen on the anthers of mature buds and quite copious amounts a few hours after anthesis. It is therefore obvious that self-pollination can take place within a flower at the time the stigma is ringed by anthers and that this can occur without insect visits.

Pollen is quickly dispersed and its presence after the second day after anthesis was only detected by microscopic examination. By this means, its presence in small amounts was noted, in dry weather, up to the fourth day. In bagged flowers traces were found up to the seventh day and up to the eighth day on flowers placed indoors in water (p. 63).

When the operculum falls, the stigma is rather pointed in outline and darker green than the style. Within the next three or four days the trend is towards a more rounded shape and a lighter colour and in many cases the latter features develop still further until, about the sixth day, the stigma may be very swollen, shining white and sticky in appearance. The period for which this condition persists varies considerably from about two days to a week, then the stigma develops a brown speckled appearance and the style normally withers after some 11 to 20 days.

In some cases, especially in G6, the stigma may fail to develop a receptive appearance and it has been noted to persist for up to three months in a yellow or pink condition. The same applies to out of season winter flowering at the tree bank.

Nectar collects at the base of the floral cup during the first three or four days after anthesis. Its presence is most obvious in bagged flowers, but much less so in flowers exposed to the effects of rain and insect visits.

5.3 Anatomy related to phenology

The timing of flower primordia initiation and other anatomical features was examined, including that of microsporogenesis, ovule structure and distribution of the ovules and ovulodes. Observations were related to the three main stages of flower bud development already described and they may thus provide a time scale framework on which to base more detailed study on specific subjects.

Methods

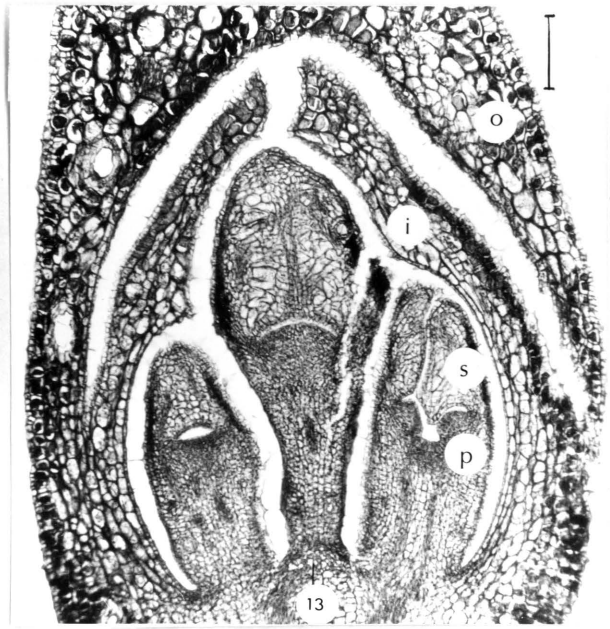
Starting with buds at their earliest recognisable stage, collections were made from the clone G15 every five to 20 days, over a period of about four months during the development of the bud.

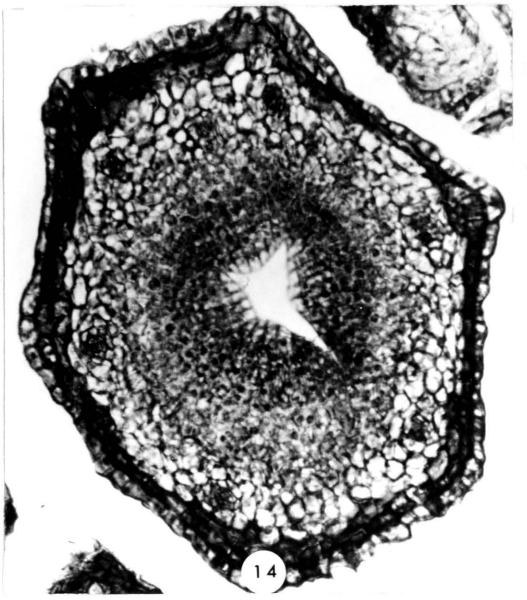
In view of the remarks by Davis (1968) about difficulty of fixation and paraffin infiltration, the buds were first prepared by making thick longitudinal or transverse sections by hand. The material was then fixed in F A A for one or more days, dehydrated with dioxane, embedded in paraffin for a period of up to two weeks, sectioned at 10 µm and stained in hematoxylin.

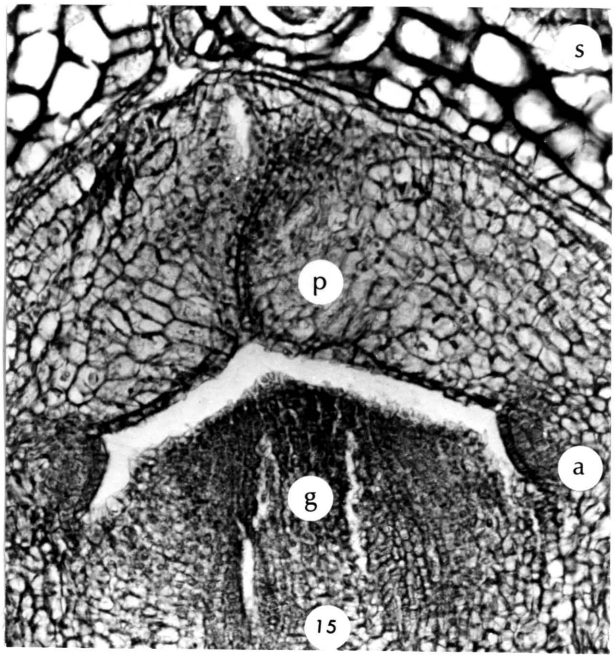
Initiation of flower primordia

By the time the umbel buds had become recognisable as such, the sepaline operculum had already developed, with basal lobes of the type illustrated by Pryor & Knox (1971). The petaline primordia had also been initiated at this stage and in transverse section, four growth centres were seen to have developed (Figs. 13 and 14). The initiation of the petals is interpreted as taking place shortly after that of the sepals in a manner similar to that described by Pryor & Knox for some of the other species in the subgenus *Symphyomyrtus*.

Anther primordia were observed at the base of the petals some ten days later, with a structure which later forms the style well developed and showing five growth







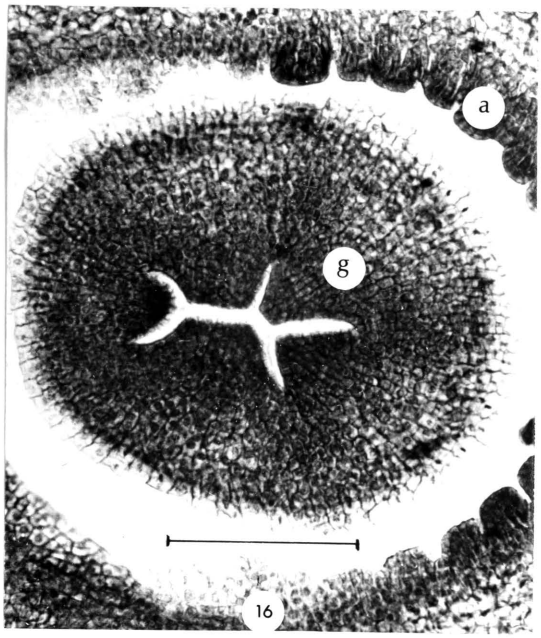


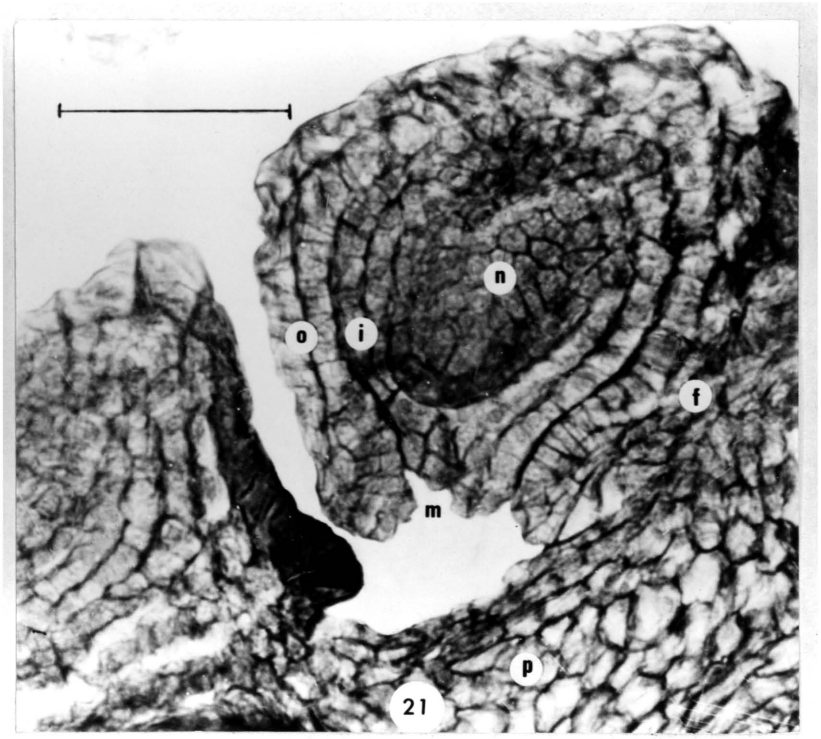
Fig. 13. Longitudinal section of an umbel bud when it first becomes recognisable. Three flower buds enclosed in inner and outer bracts (i and o); bud on the right with sepaline operculum (s) and initials of the petaline operculum (p).

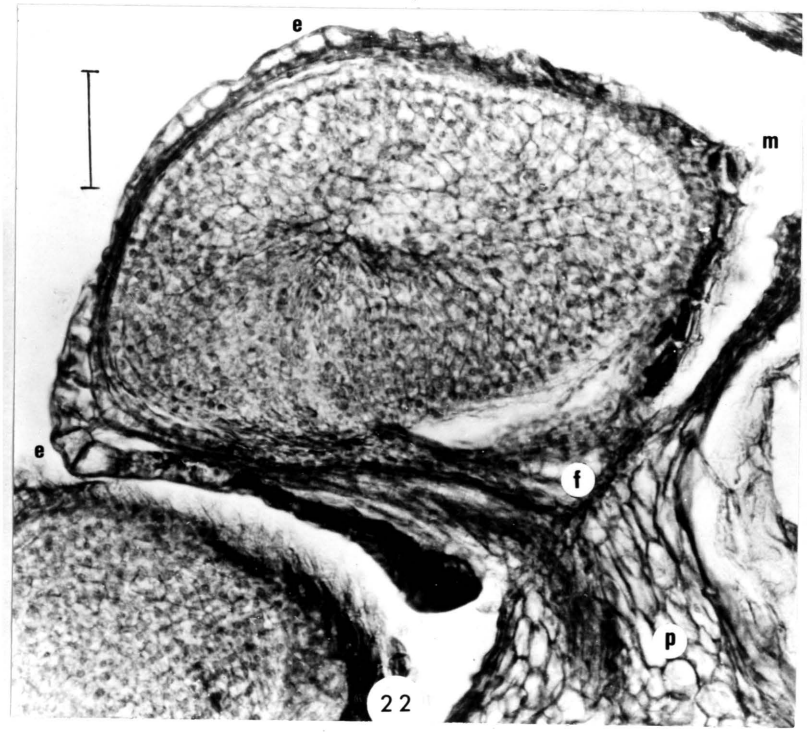
Fig. 14. Transverse section of a flower bud at about the same stage as the above, showing the sepals and inner corolline ring with four growth centres.

Fig. 15. Longitudinal section of a flower bud some ten days later than the above showing initiation of the anthers (a) and of the style (g).

Fig. 16. Transverse section of a flower bud at about the same stage as Figure 15, showing initiation of the anthers, and of the style with five growth centres.

Bars represent 100μm.



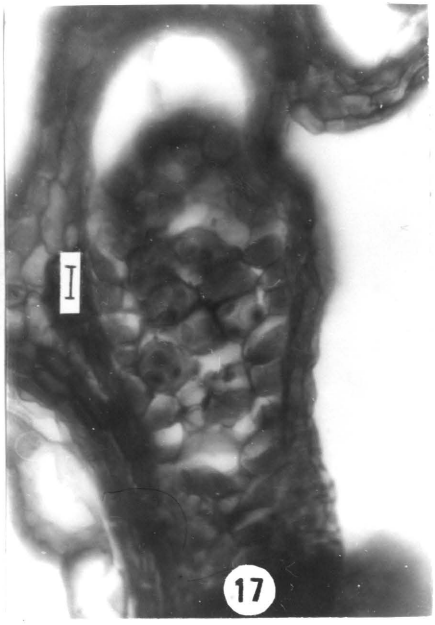


Figs. 21 and 22. Portions of transverse sections of flower buds showing ovules.

Fig. 21. An anatropous ovule some three weeks after bract shed.

Fig. 22. A hemitropous ovule some three weeks later than the above, as the outer operculum of the flower bud is being shed.

Enlarged cells of outer integument (e), inner and outer integuments (i and o), funicle (f), micropyle (m), nucellus (n) and placenta (p).
Bars represent 50 μm.



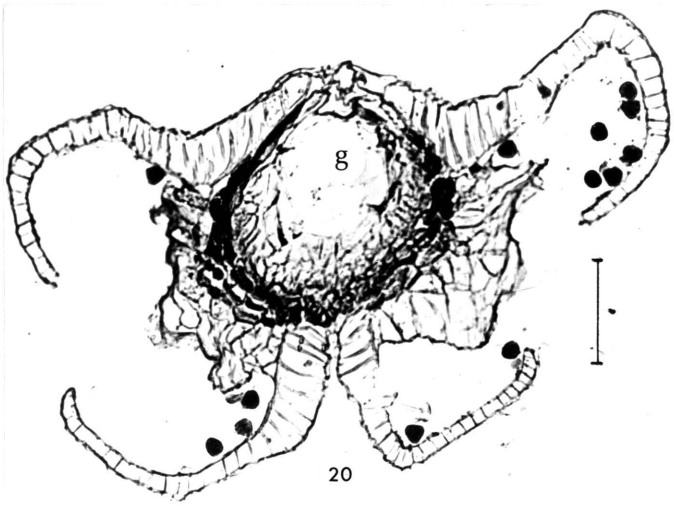


Fig. 17. Portion of section of an anther showing the microspore tetrads. Bar represents 10 µm.
Fig. 18. Longitudinal section of an anther with newly liberated microspores.
Fig. 19. Dorsal view of anther showing filament inserted below the large dorsal gland (g).
Fig. 20. Transverse section of a dehisced anther with dorsal gland. Bar represents 100 µm.

centres in transverse section (Figs. 15 and 16). Ovule and ovulode primordia were seen some 30 days later, i.e. when the time for bract shed was approaching.

These changes conform to the sequential development of the sepals, petals, anthers and gynoecium referred to by Pryor & Knox (1971), except that in this case, the formation of the style in relation to that of the anthers appears to be early, by comparison with illustrations of related species.

Anther development

Some three weeks after their initiation, the anthers start to become differentiated from the filaments. Meiosis and the formation of microspore tetrads were observed some three weeks after bract shed, in buds with two opercula still present, but before the outer operculum had become discoloured prior to shedding. The tetrads are tetrahedral (Fig. 17) and when they become separated, some exine is already deposited on the walls of the microspores and three germ pores are visible (Fig. 18). All these changes from meiosis onwards appear to take place in a short space of time and were seen in sections of a single flower.

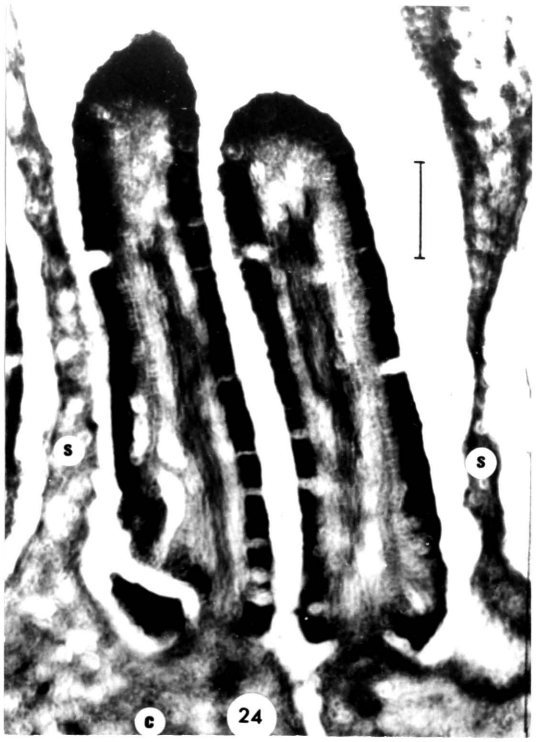
The anthers are rather more distinctly obovate in shape than the illustrations and description by Blakely (1955) for the section *Macrantherae* and the subsection *Longiores*. But there is conformity in other respects, the anthers being versatile, with a large dorsal gland below which the filament is attached to the connective at or below the centre. There are two bisporangiate lobes extending the whole length of the anther and dehiscence is by longitudinal slits (Figs. 18 to 20).

The gynoecium

The ovary contains numerous ovules and infertile ovulodes, the development of which is best observed in transverse sections.

Some three weeks after bract shed, i.e. at about the same time as the formation of the microspore tetrads, two integuments, each two cell layers in thickness, were observed to have grown round the ovules. These vary from anatropous to hemitropous (Figs. 21 and 22). The ovules are so orientated that the micropyle is on the side of the ovule facing the nearest septum. Differentiation of the megaspore mother cell was observed shortly after the integuments had grown round the nucellus.

As the ovules develop, most of the integument cells become flattened, although certain groups of cells of the outer integument become enlarged, or retain their size,



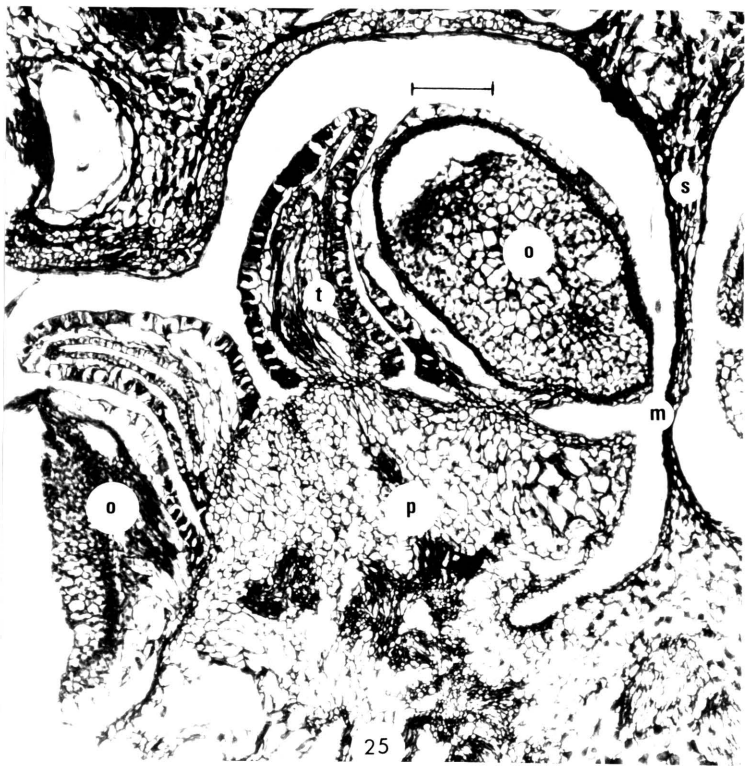


Fig. 23. Transverse section of an elongated ovulode showing signs of a double integument round the nucellus (n).

Fig. 24. Portion of longitudinal section of the ovary showing two elongated ovulodes at the top of the placental column (c) and two septa (s).

Fig. 25. Portion of transverse section of the ovary showing two truncate ovulodes (t) flanked on either side by two ovules (o). Placenta (p), and micropyle (m).

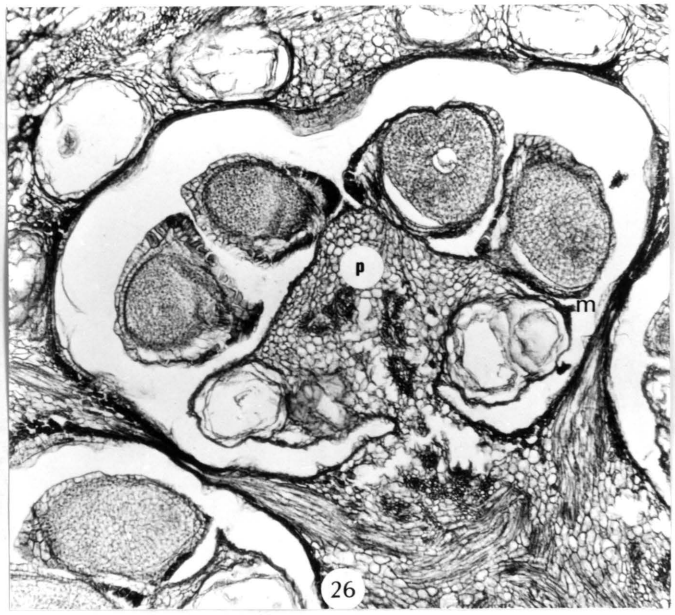
Bars represent 100 μm.

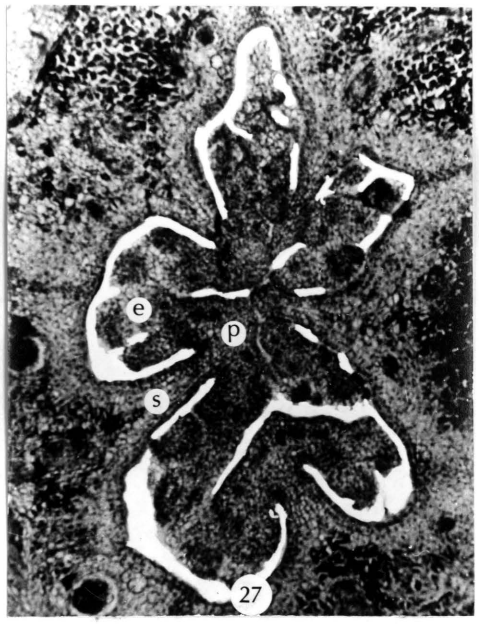
and project somewhat beyond the surface of the ovule, as seen in Figure 22, which is from a flower as the outer operculum is being shed. The micropyle becomes indistinct at this later stage, although its position may be deduced from the earlier observations, from which it is seen to be associated with one of the above mentioned projections. The outer layer of integument cells is deeply stained in the region between the micropyle and the funicle and this contrasts quite distinctly with the opposite or chalazal end of the ovule where the corresponding cells are larger and still more deeply stained, as seen in Figure 22.

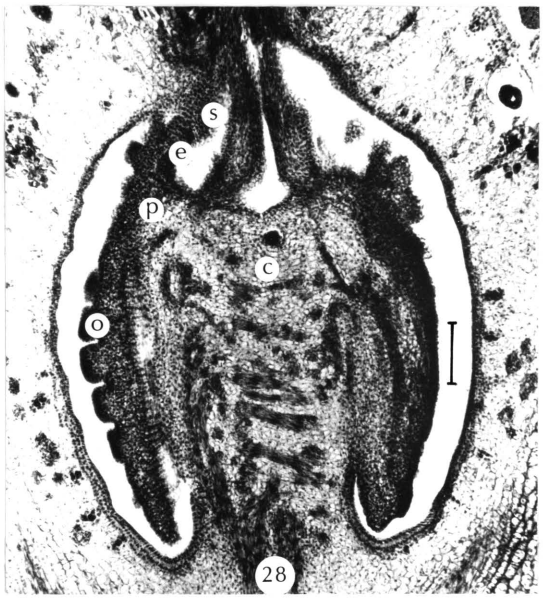
Two types of ovulodes are recognised. The elongated type are so termed here because they are much longer than they are broad and because they are identifiable, from initiation onwards, by their position round the top of the placental column. They give rise to the elongated chaff particles in the ripe fruit (p. 44). In transverse sections of flowers, taken some three weeks after bract shed, the cells at the distal end of these ovulodes sometimes appear to have a double integument (Fig. 23), but in by far the majority of cases, only a single outer integument can be detected. This consists of a single layer of cells which soon becomes capable of taking a very deep stain. These features are illustrated in Figure 24 which is from a flower bud which has just lost the outer operculum. Within this integument, rows of square cells often develop, typically two in number, arranged down one side of the ovulode. The inner nucellus tissue degenerates into large thin walled elongated cells.

The second type of ovulode occurs in the lower half of the ovary mixed with the ovules and they are referred to as truncate ovulodes because they give rise to the sterile particles referred to as truncate chaff. All structures in the lower part of the ovary initially develop two integuments and it is difficult to distinguish between the truncate ovulodes and the ovules with which they are mixed. Later, these ovulodes develop in a similar manner to that described for the elongated type, including the formation of rows of square cells, although the outer layer of cells does not become so densely stained. These features are illustrated in Figure 25, which is from a flower five days after anthesis.

The ovules and truncate ovulodes in the lower half of the ovary typically form four vertical rows which sometimes consist of two adjacent rows of ovulodes, flanked on either side by a row of ovules (Fig. 25), although this pattern does not occur consistently. Apart from the fact that the numbers are reduced to two per locule at the







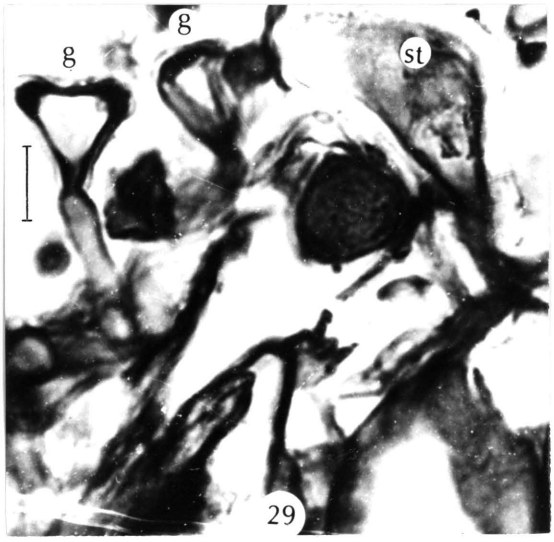


Fig. 26. Portion of a transverse section of the ovary, showing a locule with four ovules, as compared with the arrangement of ovules and ovulodes in Figure 25. Micropyle (m), placenta (p).

Fig. 27. Transverse section at the top of the ovary above the level of the placental column of a flower three weeks after bract shed. Placenta (p), elongated ovulodes (e), septum (s).

Fig. 28. Longitudinal section of the ovary of a flower at the time of bract shed, showing lack of a placental column at the top of the ovary. Placental column (c), ovules or truncate ovulodes (o), placenta (p), elongated ovulodes (e), septum (s). Bar represents 100 µm.

Fig. 29. Portion of longitudinal section of a receptive stigma. Papillose surface stigma cell (st), germinating pollen grains (g). Bar represents 10 µm.

base of the ovary, the relative arrangements of the ovules and ovulodes have been observed to vary, even in different locules of the same transverse section. In addition to the pattern shown in Figure 25, there may be four ovules (Fig. 26), or three ovules and one ovulode, although the latter have always been seen to occupy a central position, not a flanking one.

A common feature of all these structures is the very short rather broad funicle. In buds with the outer operculum being shed, embryo sacs were observed at the two nuclear stage in those structures which had been classed as ovules.

The placentation in the (usually) five-locular ovary is axile (Fig. 26) except in the upper half where there is no placental column and the partitions between the locules are incomplete. There is a resemblance to the free central type of placentation here, except that the septa extend upwards to the top of the ovary (Figs. 24 and 28), gradually increasing in the thickness till they merge with the top of the ovary; later, in the ripe fruit, they form a "keel" which emerges from the centre of the inner surface of each valve. In the young flower bud, the upper parts of the placentae, bearing elongated ovulodes, are seen to be attached to the lower parts of the septa where these join the top of the placental column (Figs. 27 and 28).

The inner walls of each locule are lined by an epidermis with a distinct cuticle, and oil glands are conspicuous in the placenta and in the ovary wall. The style is solid and the surface cells of the stigma become papillose at the receptive stage (Fig. 29).

5.4 Onset of flowering

For the sake of early seed production in seed orchards, various means have been adopted to promote flowering at the earliest possible age, e.g. by the application of fertilisers, and by grafting. But from local experience and from observations elsewhere (e.g. Eldridge, 1964) there appears to be no need for such action in eucalypts.

In the seed orchard, out of 120 surviving ramets of the five main clones which were planted in January 1966, 94 flowered during 1968, i.e. some three years after sowing the seed for stock plants. This applied where the scions were taken from the crown of the selected tree, but where they were taken from coppice shoots, flowering was found to be delayed for a year or more.

A limited amount of information available from routine records in a site near the tree bank enabled a comparison to be made of the onset of flowering of grafted

Table 3

Dates of nursery operations and of subsequent first flowering; periods before flowering by clones in grafted plants, seedlings and cuttings

	Dates and periods for				
	Grafts clones G6, 10 & 19	Seedlings clones G6, 15, 17 & 19		Cuttings clones G15	Cuttings clones G19
Seed sown	ca. 4/66	ca.9/68[1]	ca. 9/68[2]		
Cuttings set				11/65	3/65
Grafted	1/67				
Planted	4/67	1969	1969	12/66	12/66
Flowered	2/69	2/71	2/71	12/67	12/67
Period (years)	2½–3[3]	2½[4]	2½[5]	2[6]	2½–3[7]

[1]Cross-pollinated [2]self-pollinated.

[3] to [7]	[3]	[4]	[5]	[6]	[7]
Number planted	11	130	130	1	1
Number flowered	8	4	5	1	1

Table 4

Monthly record of number of ramets in flower by clones 1967/68

Clone	Total Nr. of ramets	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jne.	Jly.	Aug.	Sep.	Oct.
G6	12					4	7	7	6	1	2		1
G10	26	1	6	8	10	10	22	26	22	8	4	4	3
G15	14	2	3	3	3	4	12	13	13	4	4	7	5
G17	23	1	4	3	3	8	19	23	21	15	13	17	11
G19	14		4	2	4	7	14	14	14	11	8	3	2
Total		4	17	16	20	33	74	83	76	39	31	31	22

plants with that of seedlings, including those derived from self-fertilisation. This information is summarised in Table 3, where results are also included from two trees which were raised from cuttings. The entries under the heading "seedlings" take the form of a general statement covering Tests 5 to 8 of Table 19, page 82. Irrespective of the type of plant, the first flowers appeared at about the same stage, i.e. at two to three years after sowing the seed or setting the cuttings.

5.5 Season of flowering

Florence (1964) studied season and duration of flowering in a number of natural stands of *E. pilularis,* by collecting flowering components in traps, as they were cast.

Some idea of the season of flowering at the seed orchard was obtained simply by monthly counts of the number of ramets in flower, from November 1967 to October 1968, irrespective of the numbers of flowers seen: the intensity aspect was thus ignored, as were also seasonal differences. The results in Table 4 show the total number of ramets in flower among the five main clones which were established before March 1966. On this basis, the main flowering period for the season was from April to June, although there was some flowering throughout the year.

From observations of the few ramets in the tree bank, the corresponding season in that site is February to March, so that there is a clear difference with site, as may also be noted from Blakely (1955) who gives June to August for *E. grandis* in Australia.

5.6 Summary

The first flowering occurs at two to three years of age; the season of flowering varies widely with site and is mainly between February and June, although some out of season flowering occurs.

Phenological observations resulted in the recognition of three flower bud stages, each lasting four to six weeks. There is the "umbel bud" stage when the flower buds (typically seven) are enclosed in six or more bracts; after the bracts are shed, the buds at first have two opercula, then only the inner petaline operculum remains. Yellowing of the latter indicates that anthesis is imminent, and provides a useful guide indicating that the buds are at a suitable stage for emasculation prior to controlled pollination.

Anatomical observations are related to the above flower bud stages. The microspore tetrads are tetrahedral and their formation was observed in buds with two oper-

cula still present, but before the outer sepaline operculum had become discoloured prior to being shed. The ovule primordia were seen to develop as the time for bract shed was approaching and some three weeks later, at about the same stage as the formation of the microspore tetrads, two integuments, each two cell layers in thickness, were observed to have grown round the nucellus. The ovary also contains numerous infertile ovulodes and two types of these structures are recognised, the "elongated" and the "truncate" ovulodes.

The material examined shows similarity to related species in regard to the relative timing of initiation of the perianth whorls (Pryor & Knox, 1971), most though not all) features of the anther (Blakely, 1955), also the type of inflorescence, the emarginate shape of the cotyledons and the two independently-shed opercula of related species (Johnson, 1972). However, the hemitropous condition referred to by the latter is not typical of the ovules examined and the truncate ovulodes and ovules are not always arranged on the placenta in as regular a manner as might be expected from the descriptions by Carr & Carr (1962b) for certain other species.

Chapter 6

THE FRUIT, SEED AND SEEDLINGS

Some of the subjects dealt with here are included because of their relevance to Chapters 7 and 8, while others are of more general interest.

6.1 The fruit

The fruit is a capsule, opening by valves which begin to form a month or so after anthesis, when radial splits appear on the surface of the top of the ovary. These extend inwards from its perimeter, but do not usually quite meet at the centre. They demarcate the margins of the valves of the mature fruit which are usually five in number and which alternate with the five locules of the ovary. Once these splits have formed, the valves are capable of opening, even though the seeds are immature at this stage.

As has been found for other species in the genus (Cremer, 1965; Christensen, 1971a), opening of the valves occurs on drying and is accompanied by widening of the locules and rupturing of the walls, followed by seed shed. The valves of dried capsules can be made to close again by placing them in water. These changes occur as a result of the action of rings of hygroscopic tissue in the locule wall (Figs. 11 and 12, facing p. 29).

6.2 Capsule maturity

It is necessary to know when to reap open pollinated seed, for example in a seed orchard, but because of the wide range in time of anthesis, a known maturation period is of limited use. An additional basis of assessment was therefore sought and capsule colour was tried as a criterion; seed and chaff colour were also observed.

Methods

The five main clones were tested in the tree bank in 1966 and in the seed orchard in 1967. Flowers at comparable stages of development were labelled and the capsules were taken randomly for reaping at various periods thereafter. Notes were taken on the appearance of the capsules and seeds at each reaping and the seeds were placed for testing in porous germination dishes.

Table 5

Capsule maturity based on numbers of viable seeds per capsule and on colour of capsules and of seeds after various periods from anthesis in the tree bank 1966

Months from anthesis	Colour of capsules	Colour of seed	Mean numbers of viable seeds per capsule in clones G6	G10	G15	G17	G19
2,9	g	y–br	–	–	–	–	–
4,7	g–br	l–mbr	7,0	8,7	7,9	1,2	3,9
5,6	g–br	dbr	7,4	9,8	11,4	5,8	5,8
6,2	g–br	dbr	2,8	13,0	9,8	2,2	5,0

Basis, 12 capsules per clone at each age except G17 6,2 months, which was from 6 capsules only.

Key to colours in tables 5 and 6

capsules: g green, br brown, g–br shows a range in colour among the capsules, gbr general appearance mottled green and brown.

seed and chaff: y yellow, bl black, l light, m medium, d dark, y-br shows a range in colour among the seeds, ybr yellowish brown.

Table 6

Capsule maturity based on numbers of viable seeds per capsule and on colour of capsules, of seeds and of chaff after various periods from anthesis in the seed orchard 1967

Months from anthesis	Colour of capsules	Colour of seed	Colour of chaff	Mean numbers of viable seeds per capsule in clones G6	G10	G15	G17	G19
5,3	g–gbr	y–ybr	y–ybr	4,6	3,9	1,7	0,3	1,9
6,3	g–gbr	br	lbr	8,1	2,7	4,2	0,8	0,5
7,2	g–br	br–bl	lbr	11,4	3,3	1,6	1,3	1,4
8,2	gbr	br–bl	l–mbr	11,0	3,2	4,9	1,2	1,1

Basis 10 capsules per clone at each age.

Results

It is evident from Tables 5 and 6 that seed from the tree bank became viable at between 2,9 and 4,7 months after flowering and that from the seed orchard before 5,3 months. However, since the seed from the two sites darkened further in colour by 5,6 and 7,2 months after flowering, this was taken as indicative of a process of further maturing. By this time the capsules had started to turn brown and for the purpose of reaping after unknown dates of pollination, these seed and capsule colours were regarded as suitable guides to maturity. Where pollination dates were known, periods of at least five months and seven months after flowering were considered suitable before reaping should be done in the two sites.

No significance was attached to the slight changes in chaff colour, neither was there a steady change in number of viable seeds corresponding with fruit age, as demonstrated by Pawsey (1965) for *Pinus radiata.*

In the tree bank, a single generation may be completed in as little as three to four years. This period consisted of two to three years from sowing the seed to appearance of the first flower buds, four months for development of the flower buds and six months for maturation of the seed.

6.3 Parthenocarpy

The question as to whether parthenocarpic fruit is set was investigated in the tree bank from 1970 to 1972.

Methods

Fertilisation was prevented in one set of maturing flower buds by emasculating and bagging, and it was promoted in a similar set of emasculated buds by repeated cross-pollination "by anther", changes being made in the clones from which pollen was obtained on the various pollination occasions.

Results

The columns headed "capsules with seed" in Table 7 indicate the degree of success of the two treatments in preventing and in promoting fertilisation. Except for G6, G50 and G60, 96% to 100% of the capsules had seed after cross-pollination. As regards the prevention of fertilisation, since as many as five seeds were germinated,

Table 7

Fruit set in various clones after prevention and promotion of fertilisation.

Year / Bag Nr.	Clone	Fertilisation prevented by emasculation and bagging. Nr. flws.	Fruit set Nr.	Fruit set %	Capsules with seed Nr.	Capsules with seed %	Nr. seeds	Fertilisation promoted by pollination of emasculated flowers. Nr. flws.	Fruit set Nr.	Fruit set %	Capsules with seed Nr.	Capsules with seed %
1970												
333	G6	99	68	69	0			112	106	95	58	55
358	G10	90	10	11	0			106	104	98	104	100
300	G15	105	97	92	0			100	85	85	84	99
345	G17	116	9	8	0			105	97	92	96	99
317	G19	101	1	1	0			115	99	86	95	96
1971												
1131	G36	24	19	79	0			15	14	93	14	100
1135	G44	30	16	53	0			15	4	27	4	100
1115	G45	33	29	88	1	3	1	20	16	80	16	100
1159	G60	19	9	47	1	11	1	23	14	61	10	71
1143	G65	37	25	68	0			21	20	95	20	100
1972												
172	G4	45	7	16	1	14	2	52	47	90	9*	100
167	G22	50	0	0				46	30	65		
182	G35	52	0	0				56	25	45		
157	G38	51	0	0				52	32	62		
136	G47	37	0	0				34	25	74		
145	G50	53	32	60				49	42	86	23	55
177	G64	50	2	4	1	50	1	51	46	90	6*	100
151	G101	9	0	0				41	31	76		
		1001	324	32				1013	837	83		

*G4 Seed germinated from only 9 capsules.
G64 " " " " 6 "

G22, 35, 38, 47 and 101 germination records of pollinated flowers misplaced.

possibly some of the capsules under this heading may have been formed as a result of a stimulus from pollination or fertilisation followed by embryo failure, although there is no reason to suppose that this was a common occurrence. Prevention of fertilisation was complete in most clones.

Subject to the inaccuracies mentioned later (p. 43), the columns headed "fruit set" show the effects of the two treatments. The fruit set was usually distinctly higher after pollination (83% overall) than after emasculation and bagging (32% overall).

After emasculation and bagging, five clones, G22, 35, 38, 47 and 101 did not set any parthenocarpic fruit and G64, because of the high percentage of capsules with seed, might also be regarded as being in this class. The remaining 12 clones all set some parthenocarpic fruit, but the ability to do this varied widely from 1% fruit set in G19 to 92% in G15.

Clones G11 and G34 were included in this test, but are not reported on, since most of the flowers were lost while the styles were still in place. Subsequently it was found that the flowers were very easily removed after the pedicel had been bent and it was concluded that flowers of these clones were particularly prone to damage during emasculation and they were therefore regarded as unusable.

6.4 Capsule loss

As stated by Pryor (1954), flower buds are easily lost from failure to grow, or by accident and this happens, of course, at any period from the initiation of flower primordia onwards.

The concern here is with losses subsequent to anthesis and the effect which they may have in reducing the value and accuracy of experimental results which are based on seed yield.

One cause of loss is ordinary mechanical damage, especially through the action of wind, which results mainly in broken branches. Hail has a similar effect, though usually only the smaller branches, peduncles and pedicels are affected.

Another type of loss is due to die back of branches from the tip, noted especially in G10 and associated no doubt with an unthrifty condition of the grafted plant. Premature opening of capsules, to which G15 and any capsules hit by hail are especially subject, is yet another case.

The above types of loss are classed together as mechanical damage, which is recognisable in the field provided the evidence remains available. In the case of these types

of damage, no harm is done to experimental results, except that the number of capsules available for testing is reduced.

There are in addition cases such as that reported by Nienstaedt (1956) with *Castanea*. A flower may fall only a few days after emasculation and while stigmas are still receptive. Lack of fertilisation can hardly be the cause and it is much more likely that mechanical damage from twisting or bending of the pedicel during emasculation is at least a contributory cause of this flower loss. This is most common in certain clones such as G34 and G11 and individual inflorescences or flowers in other clones also appear to be more susceptible than others. Because in such cases there is uncertainty in distinguishing between early flower losses caused by mechanical damage, and losses occurring later and attributable to other causes, inaccuracies in results may occur (p. 22).

If there was no sign of mechanical damage, losses were regarded as abscissions. They may be complete, as in the case of some of the bagged clones in Table 7 (p. 41), or less complete, though probably of the same type, when certain treatments have been applied. Examples of the latter are self-pollination, also pollinations done at stages of low receptivity (Table 14, p. 70). In the latter case, there were fewer seeds per capsule after pollinations done early than at maximum receptivity and a positive correlation was found between numbers of seeds per capsule and percent fruit set:—

Table 14, receptivity Test 10 (G19x15) $r = 0{,}86$ $P = < 1\%$

" " " " 9 (G17x19) $r = 0{,}65$ $P = > 5\%$

The above Test 9 is only just short of significance at the 5% level and judging from the above and from the results of Table 7, when treatments gave nil or reduced seed yields, there tended to be a corresponding reduction in fruit set, probably related to lack of fertilisation.

6.5 Seed and chaff

The seed is always mixed with particles known as chaff, some of which may consist of small particles derived from unfertilised ovules, but the greater part is from infertile ovules.

In distinguishing between seed and chaff of the latter type by means of a hand lense, there is clearly a difference in colour, at least in fresh capsules, but the difference in size mentioned by Larsen (1965) for *E. grandis* is less obvious, because of the

wide range in size of both seed and chaff. This is confirmed in Table 9 (p. 48), where it is seen that all gauges of sieves, except the smallest, had both types of particles.

Shape provides another possible means of distinguishing seed from chaff since apart from certain indeterminate particles, three quite distinct types may be recognised on this basis. There are those which have at least one surface rounded in outline; these are taken to be seed even though there may be somewhat angular edges on other surfaces. Then there are the particles with an angular outline, which are taken to be from infertile ovules; these are of two types referred to as elongated chaff and truncate chaff, certain characteristics of which, in the flower bud stage, are described on page 32.

When separation has been done on the above bases, followed by placing of the different types for germination, it is of interest to see to what extent the anticipated germinability, or lack of it, is realised. The results of such a test are summarised below, where the large and small particles recorded refer to sizes greater than and less than 500 µm respectively, and where percentage germinations are shown for the types recognised:–

1	100 large dark coloured particles	:	taken to be seed	98%
2	72 medium size dark particles	:	taken to be seed	90%
3	16 large to medium size, but lighter in colour, more wrinkled or more angular	:	uncertain	63%
4	17 small size, dark colour but wrinkled	:	unlikely to be viable	24%
5	132 elongated, angular, light colour	:	elongated chaff	0%
6	150 truncate, angular, light colour	:	truncate chaff	1%

The distinction between viable seed and chaff was, from the above, almost certain in the case of the chaff in items 5 and 6, and 98% to 90% correct for identification of viable seed in items 1 and 2. There remain items 3 and 4, which form only a small proportion of the whole and which could not be classified with any certainty on these bases.

6.6 Sieving of seed

Working with various *Eucalyptus* species, Andrade (1961) made use of sieves, apparently of various sizes, to separate seed from chaff, or at least to obtain a higher percentage purity of the former.

Endecotts test sieves, B.S.410, were used to investigate several aspects of sieving *E. grandis* "seed"*, through eight gauges varying from 1000 to 300 µm. Two tests were done. In the first or "germination test", replicated seed samples were taken in which the main object was to ascertain, for each gauge, the numbers of seeds and their germination time, also subsequent survival and heights of the seedlings. An estimate of seed weight, all gauges combined, was also made.

In the second, or "weight test", the same seed source was used, to get approximate (unreplicated) values for weights of seed and of chaff and the resulting percentage purity by weight, in this case for each gauge.

Methods

For the purpose of the germination test, twenty five capsules, approximately one year old and derived from open-pollination, were collected in the tree bank in 1971 from each of twenty clones.

After extraction, the "seed" was dried and weighed. It was then mixed and divided into ten approximately equal portions by the random cup method (I.S.T.A. 1966) and the samples so obtained were made equal to each other by weight. These were then sieved and the 80 fractions were placed separately in the Jacobsen apparatus for noting numbers germinated and the germination time (p. 84). After germination, the remaining chaff was bulked, then dried and weighed as before.

Seeds were removed as they germinated and approximately 50% of them were taken randomly from each pad and were allocated randomly to nursery boxes, where they were drill sown 5 cm apart. Heights of seedlings were measured in the nursery to the nearest ½ cm, from ground level to insertion of the highest leaf pair. This and a count of survival was done at age 4½ months after the seed had been placed in the germinator.

For the weight test approximately 20 open pollinated capsules were collected from each of the same clones, giving a total of 418 capsules in all. After extraction, the "seed" was sieved and the contents of each gauge were dried, then weighed and placed for germination. When this was complete the remaining chaff was dried and re-weighed to obtain the weight of seed for each gauge by subtraction.

---

*In this section, "seed" refers to seed and chaff, but the omission of the inverted commas indicates that chaff is excluded.

Table 8

Numbers of seeds from 500 capsules, germination time, survival and mean heights of seedlings, according to seed size (germination test)

Gauge µm	Seeds actual nr. ±S.D.	Seeds nr.	Seeds effective[1] %	Seeds R.T.[2]	Germinating seeds & seedlings germin. time days	sown nr.	survivals nr.	%	mean height cm
1 000	55±2	41	2		4,95	24	18	75	10,4
850	516±19	310	13	15	5,32	227	135	60	9,6
710	1419±16	724	30	45	5,97	693	353	51	8,5
600	1609±11	837	35	80	6,76	800	416	52	8,8
500	772±8	332	14	94	8,13	393	167	43	7,0
420	303±8	139	6	100	11,01	144	66	46	7,3
355	36±2	7			12,67	16	3	19	5,7
300	1	0			10,0	1	0	0	
	4 711	2 390							

[1]Effective number of seeds, shows the actual number of seeds reduced by the % survival of seedlings.
Effective % shows the effective number in each gauge expressed as a percentage of the total.
[2]R.T. Running total.

In both tests, the "seed" was spread evenly on the germination pads and as near as possible at comparable density in each case, the maximum weight per pad being about 100 mg. All drying before weighing and re-weighing was done for a period of six days in a desiccator containing silica gel.

Results

For the germination test, the total number of germinations and the mean germination time of gauge 420 may have been affected a little as a result of a mishap which occurred with one germination pad (out of 30), although the seeds were collected and replaced as well as could be arranged.

Shortly after emergence above ground level, 3% of the seedlings (mostly from the larger seeds) were killed by an agency (possibly insects) which caused removal of the cotyledons. Since one type of stock was affected more than the rest, all such seedlings were excluded from the numbers recorded in Table 8 as having germinated.

It appears from Table 8 that the smaller seeds had a longer germination time, also that the seedlings derived from them had poorer survival and less vigorous early height growth, and there was confirmation of this from the following strong correlations which were obtained:—

Seed size and	germination time	$r = -0{,}894$	$P = < 1\%$
. .	survival	$r = +0{,}905$	$P = < 1\%$
. .	mean height	$r = +0{,}949$	$P = < 1\%$

The data in Table 9 show that the percent purity by weight also decreases with seed size, except for gauge 1 000, in which the purity was much reduced as a result of the presence of valves which became detached during drying.

Mean weights of individual seeds are also recorded in Table 9, but (from sub-column 1) it is seen that those for gauges 420 and 355 gave anomalous results in comparison with other gauges, although when the test was repeated, virtually the same weights were obtained. Therefore, as a check, some individual seeds were identified, dried and weighed, then germinated to test viability. As a result, the weights per seed obtained by this method (sub-column 2 of Table 9) were comparable with those obtained by the original method (sub-column 1), except for gauges 500, 420 and 355. Regarding the last two, the mean weights of five seeds in gauge 420 and the weight of one seed in gauge 355 were 0,1 and 0,06 mg respectively. Since these weights fit in better with those of the bigger gauges, it is concluded

Table 9

Weights of seed and of chaff from 418 capsules, % purity and weights per seed, according to seed size (weight test)

Gauge µm	Nr. seeds	seed & chaff g	Weight chaff g	viable seed g	% Purity by weight	Mean weight[1] per seed 1 mg	2 mg (nr.)
1 000	35	0,194	0,147	0,047	24	1,34	
850	446	0,267	0,051	0,216	81	0,48	0,43(43)
710	1 315	0,571	0,138	0,433	76	0,33	0,3 (21)
600	961	0,817	0,584	0,233	29	0,24	0,22(21)
500	483	1,946	1,841	0,105	5	0,22	0,14(12)
420	335	3,764	3,584	0,180	5	0,54	0,1 (13)
355	39	0,933	0,921	0,012	1	0,31	0,06(5)
300	0	0,094	0,094				
	3 614	8,586	7,360	1,226			

[1]Mean seed weight column 1 from the method originally adopted (p. 45).
ditto 2 from individually identified seeds.

The weight of seed and chaff in gauges 1 000 to 500 inclusive, totals 3,795 and that of viable seed totals 1,034 g.

that they represent more correct results than those by the original method in the case of these two gauges.

The total dry weight of "seed" in the germination test was 10,025 g and the chaff remaining weighed 8,344 g, leaving 1,681 g representing the weight of viable seed. Incorporating these figures with those in Tables 8 and 9 we have the following:–

	Germination test	Weight test
seed weight	1,681 g	1,226 g
"seed" weight	10,025 g	8,586 g
chaff weight	8,344 g	7,360 g
number of seeds	4 711	3 614
number of capsules	500	418
mean seed weight	0,36 mg	0,34 mg
weight of "seed" per capsule	0,02 g	0,02 g
ratio by weight of seed:chaff	1:5	1:6

No gauge was without impurities and clearly it was not feasible to obtain pure seed by sieving, but certain fractions could be discarded in such a way that, although a lower total number of seeds would be obtained, those retained would include the best as regards survival, and as regards initial vigour as represented by germination time and early height growth. It is also seen from Table 9 that bulk could be reduced and purity improved by sieving. For example, if all gauges smaller than 500 were discarded, weight of "seed" would be reduced by over 50% from 8,586 g to 3,795 g while retaining 1,034 g out of 1,226 g or 84% of the weight of seed (Table 9, footnote referring to the third and fifth columns).

6.7 Seed viability

It is recognised that *Eucalyptus* seed remains viable for many years (Larsen, 1965). Some seed was put into storage in December 1966, but without any intention of including results in the present work. No results of germination tests are available for the seed when it was originally stored and therefore no formal test can be reported. Nevertheless, it now seems worth while to record results of a germination test done in December 1973 after a seven year period.

Originally, there were three different storage conditions, (i) in a stoppered glass jar at room temperature, (ii) as (i) but with silica gel and (iii), as (ii) but at 4 degrees C.

The seed was tested in December 1973 by taking five 0,1 g samples by the random cup method and placing them for germination in the Jacobsen apparatus.

The numbers of germinations per kg after the seven year period are compared below with a routine viability test of fresh seed collected in 1973 (iv), with the following results:–

(i)	mean of 5x0,1 g samples	476 000	seeds per kg
(ii)	ditto	618 000	ditto
(iii)	ditto	684 000	ditto
(iv)	mean of 20x0,1 g samples	512 000	ditto

Although the test is not a controlled one because of the absence of results at the beginning of the storage period, it does appear that there was little or no loss of viability over a period of seven years and that the use of a drying agent and low temperature had a beneficial effect.

6.8 Seedling development

When observed under laboratory conditions, the typical sequence of events in germination was splitting of the testa, followed by emergence of the radicle, the pink hypocotyl and the cotyledons, in that order. The young seedling is normally erect on the germination pad a day or so after emergence of the radicle and by this time the first root hairs have appeared in the form of a ring round the radicle, referred to by Gauba & Pryor (1958) as the clinging disc. The testa or part of it may still partly enclose the cotyledons at this stage. Elongation of the hypocotyl continues for a week or more after germination and during this time the cotyledons, which are petiolate and emarginate (Fig. 30, facing p. 52), reach their full size.

Minor irregularities noted in the above include poor development of the radicle and colour deficiency in the hypocotyl and in the cotyledons; the latter may at first be a pale yellow, though this becomes normal in a day or so. In seedlings apparently lacking in vigour, the cotyledons may also tend to remain enclosed in the testa, especially in the case of germinations from small seeds (p. 47).

After germinating seeds had been transferred singly from the germinator to soil, it sometimes happened that, on emergence from the soil a few days later, two seedlings appeared. Since each typically had a separate testa attached to the cotyledons it was concluded that the extra seedling resulted from accidental transfer of a second minute seed. However, in one case out of several thousand germinations in the Jacobsen apparatus, two seedlings were seen to emerge from a single seed on

the germination pad, one of which only survived for a few days. Presumably this represents a single case of polyembryony although others may have gone undetected.

For the genus as a whole, and apart from the cotyledons, Blake (1953) distinguishes between seedling leaves (first year after sowing) and juvenile leaves or regrowth from older plants; the former are followed by intermediate leaves, then adult leaves.

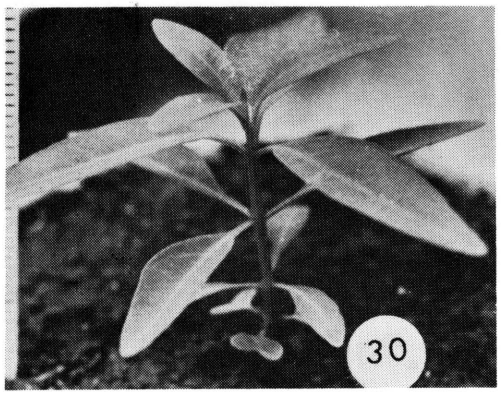
In *E. grandis*, the first formed seedling leaves are arranged in a decussate manner, the first pair appearing ten to 24 days after germination. Up to 13 pairs of this type have been noted, after which individual leaves of a pair become separated by an intranode. These leaves are quite small and are regarded as intermediate; later, adult leaves of normal size are formed.

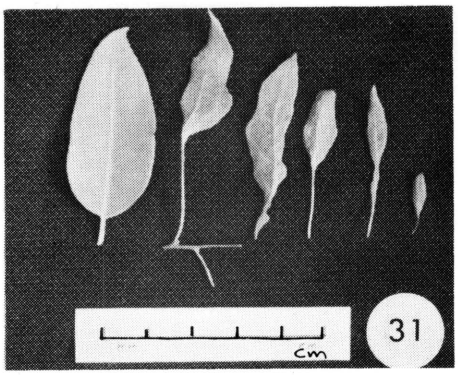
6.9 Abnormal seedlings and transplants

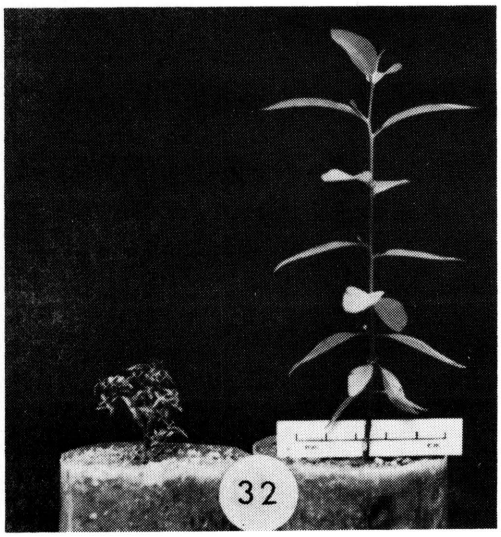
The term abnormal is used here to cover a wide range of irregularities, including certain features which may be mainly reactions to growing conditions. For example, leaves of a red colour are referred to in the descriptions which follow, but this is also a common feature in seedlings under water stress.

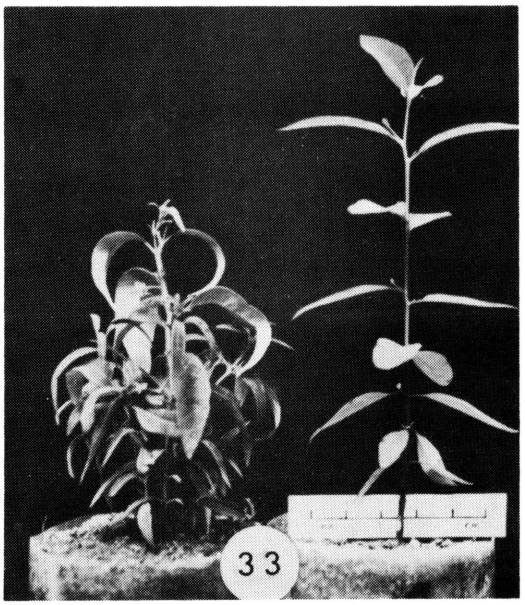
The abnormalities described below and listed in Table 10 (p. 54) were observed initially in plants raised in connection with tests of the incidence of self-fertility among clones (Table 21, p. 90), also in some of the serially numbered tests in Table 19 (p. 82). They are described here so as to record all abnormalities which were observed, from which those suitable for use in estimating natural selfing (Table 25, p. 102) were selected. The methods used in raising the seedlings were therefore as described for the above tests, to which cross references are given in the right hand column of Table 10.

Fifteen different types of abnormality were recognised and these are numbered serially in the following pages, where the condition in each case is described briefly, followed by notes on the clones concerned and where necessary the stage (laboratory, nursery, or field), the effect on vigour and survival, also whether the defect is well defined or not. The latter is entered as poorly defined, indicating difficulty in deciding whether a plant is normal or not; this is also often the case when there is a continuous range of variation, which is entered briefly as "graded series". Much the same also usually applies if a feature appears for a short while only, after which the plant reverts to normal, which condition is recorded as ephemeral.









Figs. 30 to 35. Deviant types of seedlings (Table 10).
Fig. 30. Type 1, leaves in threes. Scale in mm.
Fig. 31. Type 3, leaf margins irregular. A normal leaf is on the left and one leaf of the first leaf pair is on the right.
Fig. 32. Type 4, leaves crowded. A normal seedling is on the right.
Fig. 33. Type 5, leaves curved. A normal seedling is on the right.
Fig. 34. Type 7, leaves dark. Scale in dm.
Fig. 35. Type 8, foliage dense. Scale in cm and dm.

The abnormalities in Table 10 (p. 54) are arranged by clones, showing normal to abnormal ratios. Below, they are arranged according to the part of the plant affected. The numbered types 1 to 11 deal mainly with form and 12 to 15 with pigmentation.

Type 1 (Fig. 30)

Three cotyledons, and up to five subsequent nodes with leaves in whorls of three, after which foliage reverts to normal (Jacobs, 1955). In several clones, after self-, cross- and open-pollination. No ill effects (but see Haskell, 1961, regarding possible diagnostic value in other genera for forecasting performance).

Type 2

Cotyledons and/or first two vegetative leaf pairs deformed, with twisted blades or irregular margins, sometimes one of a pair much reduced in size or entirely absent; reverts to normal later. Mainly G58, self-, cross- and open-pollinated progeny; no ill effects; not always well defined, graded series (Mendonza, 1970).

Type 3 (Fig. 31)

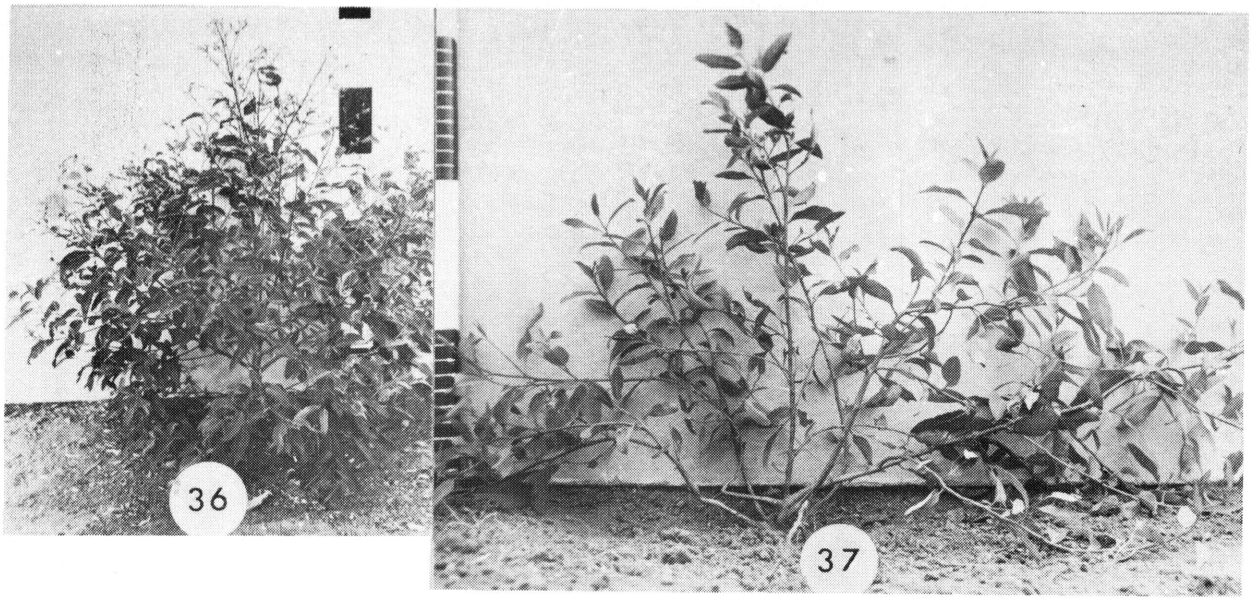
The first formed vegetative leaf pair more linear than usual, with slightly or distinctly irregular margins; later more pronounced, or leaf even reduced to a midrib; may later revert to normal. G1, self- and open-pollinated progeny, mostly in the nursery. Can survive, but vigour poor. Usually well defined.

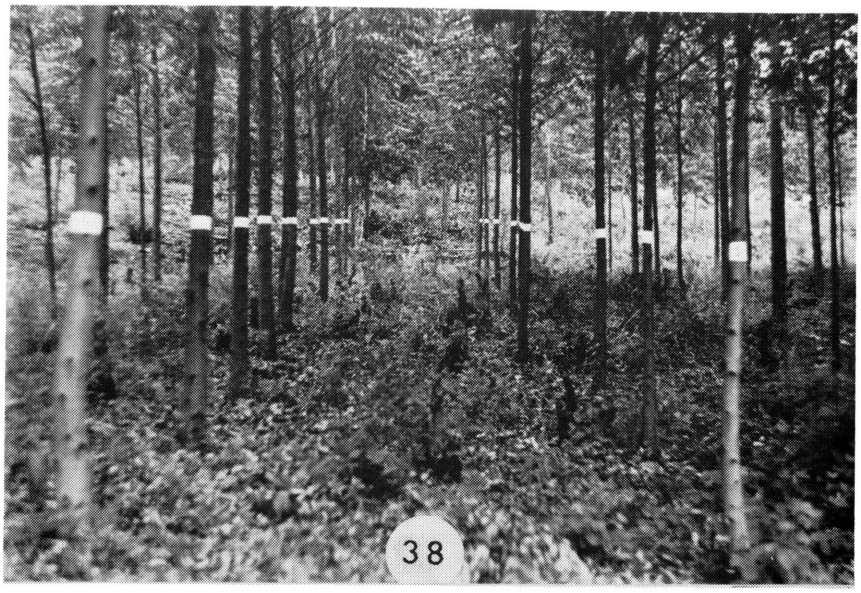
Type 4 (Fig. 32)

All leaves crowded and small, often a quarter normal length or less and with deep red colour. May revert to normal except in regard to vigour. Self- and open-pollinated seedlings of G13, 14, 24, 26, 38, 39, 61 and 64, mainly in the nursery. Plants typically dwarfed and survival poor. Usually well defined, but sometimes similar to Type 5.

Type 5 (Fig. 33)

Leaves curved downwards and occasionally slightly distorted, also initially about half normal size and rather crowded on the stem. Usually also slightly or distinctly discoloured with midrib more obviously red than usual, in some cases forming a





Figs. 36 and 37. Deviant types of seedlings.

Fig. 36. Type 9, leaves small, shed. Scale in dm.

Fig. 37. Type 10, stem leaning. Scale in dm. and cm.

Fig. 38. Inbreeding effect on diameter increment.
Line of 10 trees on left from cross-pollination, line of nine trees on the right from self-pollination, both in block 2 of Test 17 (Table 19, p. 82), mean diameter at breast height 11,5 cm and 6,4 cm respectively. Tree lines are irregular because of stumps of the previous crop.

broad line down the centre of the blade; the blade may be of a darker green than normal. Sometimes reverts to normal, or to Types 4,7 or 11. Self- and open-pollinated seedlings of G3,4, 13, 18, 29 and 30 in the nursery or shortly after planting. Poor in vigour and survival. Not always well defined, sometimes in graded series.

Type 6

Leaves of about one third normal size over the whole plant and foliage sparse. May revert to normal or develop into some other type. G15 and others, self- and open-pollinated progeny in the nursery or after planting. Occasionally distinct, but usually in a graded series (p. 51).

Type 7 (Fig. 34)

Leaves usually dark, broad and fleshy; subject to browsing by buck; some multiple stems or other stem deformity. May revert to normal. Self- and open-pollinated progeny, after planting. Poorly defined and ephemeral in G6, in G13 rather stunted and fairly well defined except in some individuals.

Type 8 (Fig. 35)

Foliage very dense and leaves rather small forming a young tree with a rounded crown; subject to browsing by buck. Gradually reverts to normal, though suppressed and with numerous persistent thin branches towards the base. G15 and G19 after open-pollination only, one tree of G9 after cross-pollination. Very distinctly different from normal.

Type 9 (Fig. 36)

Arrested development of newly formed leaves on one or more branches; the leaves typically very small, red and eventually shed, with suppression of the terminal shoot and growth of many axillary shoots. May revert to normal, but suppressed. Self- and open-pollinated progeny of G17, after planting. Rather lacking in vigour. Very distinct, or merely suspect and not differing clearly from normal.

Type 10 (Fig. 37)

Stem leaning, leaves red and rather small, later a dwarfed plant with a somewhat bushy habit and without any single upright main stem. G15, self- and open-

Table 10

Types of abnormalities in seedlings and transplants and normal to abnormal ratios by clones after three pollination treatments, cross, self and open

Clone	Type	Ratios			Cross reference	
		cross pollination	self-pollination	open-pollination	Clone/year[1]	Test nr.[2]
1	3 irregular margins					see 19,20 & 27
3	5 leaves curved		10:1	14:0	G3/70	
4	5 leaves curved		15:0	11:3	G4/70	
6	1 leaves in threes	179:1	126:1	121:1		5
	7 leaves dark	49:0	62:8	81:4		13
9	8 foliage dense	45:1	62:0	34:6		26
13	4 leaves crowded		24:6	24:7	G13/70	
	5 leaves curved					see 24(1972)
	11 procumbent					.. ..
	7 leaves dark					.. ..
14	4 leaves crowded		22:6	29:3	G14/70	
15	10 stem leaning					see 8,15 & 22
	8 foliage dense	46:0	42:0	44:3		8
	..	91:0	102:0	154:7		15
	..		27:0	121:4		22
	6 leaves small					see 8
16	12 albina		3:0	111:1	G16/70	
17	9 leaves small, shed	84:0	46:2	113:6		16
18	5 leaves curved		27:1	28:0	G18/70	
19	8 foliage dense	50:0	50:0	49:1		6
	14 variegated			many:1		
23	13 virescens		15:0	253:3	G23/70	
	12 albina		15:0	254:2	..	
	12 and 13		41:0		G23/72	
24	4 leaves crowded		43:5	31:1	G24:70	
	..	73:0	23:0	43:0		23(1972)
26	4 leaves crowded		7:1	27:0	G26/70	
	..		11:0		G26/72	
28	12 albina		19:0	39:1	G28/70	
29	5 leaves curved		34:0	29:1	G29/70	
30	5 leaves curved		22:4	28:0	G30/70	
	..					see 21(1972)
	11 procumbent					.. ..
	15 leaves pale green	153:2	65:9	88:6		21
35	14 variegated			many:1		
36	11 procumbent		27:3	8:2	G36/71	
38	4 leaves crowded		27:3	9:0	G38/70	
39	4 leaves crowded		18:5	29:3	G39/71	
58	2 leaves deformed		30:20	23:3	G58/70	
	..	204:13	70:8	191:1		18(1971)
	13 virescens	227:7	95:10	188:9		.. ..
61	4 leaves crowded				G61/72	see table 25
64	4 leaves crowded		24:3		G64/71	
	..		60:0		G64/72	

[1]Clone/year refers to entries in the first column of Table 21, page 90.

[2]Test nr. refers to the tests listed in Table 19, page 82. If also used in tests of natural selfing, the ratios are not stated here, but a cross reference is made to the test numbers concerned in Table 25, page 102.

pollinated progeny, some two to eight months after planting. Rather poor in survival, very poor in form and vigour. Usually well defined in surviving plants.

Type 11

Similar to Type 10, but leaves pale green and stem typically more completely procumbent. May develop from Type 5. G13,30 and 36, self- and open-pollinated progeny in the field a few months after planting. Very poor in form, vigour and survival. Mainly well defined.

Type 12

Cotyledons colourless and seedlings classed as "albina" (Snyder *et al.*, 1966). Open-pollinated seedlings of G16,23 and 28. Very rare. Lethal.

Type 13

Cotyledons and some seedling leaves pale or yellow green. Classed as "virescens". (Snyder *et al.*, 1966). G23 open-pollinated progeny and G58 self-, open- and cross-pollinated progeny. No ill effects, often not clearly defined, graded series. (Possibly dependent on temperature conditions for development of normal pigment, Haskell, 1961).

Type 14

Variegated foliage. One case in G19 and one in G35, open-pollinated progeny. Both died after planting. Clearly defined.

Type 15

Leaves paler green than usual. Mainly G30, self-, open- and cross-pollinated progeny, after planting. No ill effects, occasionally fairly well defined, but mostly indistinct and in any case in a graded series and ephemeral.

Selfed progenies were observed in 39 of the 40 clones recorded in Table 21 (p. 90), plus the five main clones, which are not in the table. Out of these 44 clones, the progeny of 25 had some abnormality and are shown in Table 10. No doubt this proportion would have been higher had the numbers of selfed seeds been sufficient in each case.

Five of the 15 types were not seen to have any appreciable ill effects, namely numbers 1, 2, 13, 15 and sometimes number 7. The remainder were all detri-

mental in varying degrees because of poor form, vigour, or survival and of these, numbers 4 to 6, 8 to 11 and 14 were not expressed phenotypically until near or after planting and these were therefore liable to be planted without there being much chance of prior culling.

Types 1, 2, 13 and 15 were seen after crossing as well as selfing. Type 8, apart from one case after crossing, was seen only in open-pollinated progeny.

Types 12 and 13 were initially seen in progeny from preliminary tests and Type 14 in open-pollinated nursery stock, but when the various clones concerned were used in a test of natural selfing, no deviants developed and the tests were discarded. Similar cases in which an abnormality developed in one season but not in another were seen in G24 and G64, Type 4 (Table 10).

Types 4 and 5 appeared to be closely related since some plants showed some of the features of both types, also because of one apparently changing to the other. For example, in Test 21 (Table 19, p. 82), transplants were originally classed as Type 5, but some later became similar to Type 4. Also, as shown in Table 10, deviants in G13 were classed as Type 4 in 1970, but as Type 5 in 1972. Apparently much depended on the stage when observations were made and in these cases the plants were classified according to the type which developed first.

Types 13 and 15 were much too poorly defined for it to be possible to place any reliance on their classification and the same applied to Type 9.

Many of the remaining abnormalities were, for other reasons, quite unsuitable for assessment of percent selfing and most reliance was placed on Types 3, 4, 5 and 10 which were usually sufficiently well defined. Types 6, 7 and 11 occurred in conjunction with these and provided some supporting evidence (Table 25, p.102).

As seen in Table 10, certain families, e.g. G15, included more than one phenotypically dissimilar type and in such cases normal to abnormal ratios in any one type are expressed in Table 10 as if all other types were normal.

The numbers of seedlings shown in Table 10 were often too low to give reliable indications of ratios, but it seemed that some types might be controlled by a single locus and the ratios were tested against the null hypothesis of three normal to one abnormal, using Chi-square with a probability of ,05. The results are shown in Table 25 (p. 102).

6.10 Insect damage

The intention here is to record cases of attack by insects and other agencies, which have been experienced and which occurred to the detriment of experimental results in the nursery and field. Other pests are referred to in the handbook published by the Wattle Research Institute (1972).

Very young seedlings were found liable to have the stem cut below the level of the cotyledons, resulting in death. Alternatively one or both cotyledons, or parts of them, were damaged, giving the impression that they had been bitten. Although this strongly suggested insect attack, no insect was actually observed. Mice were later suspected and the placing of rat poison appeared to be beneficial.

Crickets and various types of grass hoppers caused damage by removing terminal and lateral buds in the nursery and field. There is also a beetle, identified by the Plant Protection Research Institute as *Colasposoma* species, which appeared for short periods and fed on young foliage, particularly in early summer. Trees planted at this time were liable to lose leading shoots, especially if other vegetation had been entirely removed. Karbadust powder appeared to have some effect in controlling attacks by these insects.

The roots of plants in the field were occasionally attacked at any stage by termites, killing the tree. A light dusting of the planting hole with Dieldrin (now banned) was found effective as a control.

6.11 Summary

Capsules were regarded as mature and were reaped after periods of five months and seven months in the tree bank and seed orchard sites respectively. The capsules had started to turn brown by this time and the seed (which had been viable earlier), had become dark brown.

Capsule losses were noted to occur as a result of various forms of mechanical damage, but in addition, since six out of 18 clones tested were found incapable of setting parthenocarpic fruit, lack of fertilisation is another likely cause of capsule loss, at least after certain experimental treatments.

After passing through sieves of a series of gauges, both seed and chaff were present in each gauge except the smallest, so that it was not possible to obtain pure seed by sieving. But by retaining only the contents in gauges 500 µm and over, 84% of the total weight of pure seed was retained and purity was improved.

Further, the seeds retained gave seedlings which were superior in survival and in initial vigour, while the smaller seeds, being of lesser value in these respects were proposed for discard.

In a search for deviant types of seedlings which might be of use as genetic markers in assessing natural selfing, 15 different types of abnormalities were observed in the progeny of 25 out of 44 clones examined. There were no irregularities of any consequence during germination and no progeny showed distinct and consistent chlorophyll deficiencies during early seedling development. Most of the effects were on the form of leaves and stems, ten of which were detrimental because of poor form, vigour and/or survival, and eight of these were not expressed phenotypically until near or after planting time, so that undesirable plants could not usually be discarded by culling in the nursery.

The suitability of these abnormal types as markers for the purpose of estimating selfing was often limited because of poor definition and low numbers in some selfed families. There was also a tendency for abnormalities to develop in one season but not in others and this, as well as the late phenotypic expression were further drawbacks.

Chapter 7

POLLINATION AND RECEPTIVITY

As a background to problems related to pollination and the breeding system in forest trees, investigations such as those by Archimowitsch (1949) with sugar beet and by Haskell with groundsel (1953a) and with maize (1953b), are of general interest in connection with this chapter, as well as Chapter 8. As regards eucalypts, the intention here is to check on the information on page 4 in regard to its applicability to *E. grandis* under local conditions. In the course of this, related matters are considered, especially the occurrence of selfing at and shortly after anthesis.

7.1 Pollinating agents

A considerable variety of insect types have been observed visiting *E. grandis* flowers at different times in the tree bank and about two dozen of these were forwarded to the Plant Protection Research Institute, where they were identified. The orders most commonly represented include eleven species in the Coleoptera, about the same number in the Hymenoptera and Lepidoptera and even some of the Hemiptera and Diptera.

Few of the above are regarded as important pollinators, because of their relatively sluggish habits. The "ants" in particular remain immobile for long periods, apparently taking nectar and not touching the stigma. "Hornets", "moths" and species of *Lyctus* beetles are more active and may play some part in pollination, but only the latter is common. The honey bee appears to be the most likely pollinator, because of its active habits, size and observable functions in collecting pollen and taking nectar.

Some observations were made in the tree bank in connection with the part played by insects there.

Methods

Maturing buds were taken for emasculation and for comparison of their seed yield with that from ordinary untreated buds, which were labelled at the same time. This was done over three consecutive seasons from 1966 to 1968 (labels 27, 83 and 283), and in 1970 (label 201) by a similar test with the inclusion of a third

Table 11

Numbers of seeds per capsule from emasculated flowers compared with untreated flowers

Clones 1966–1968	Numbers of Seeds/capsules used			Numbers of Seeds per capsule		
	Untreated	Emasculated	Emnet.[1]	Unt.[2]	Emas.[3]	Emnet.[1]
6	473/50	1/42		10	<1	
10	106/20	2/25		5	<1	
15	231/44	41/46		5	<1	
17	293/43	87/21		7	4	
19	96/22	15/11		4	1	
47	74/10	10/9		7	1	
50	50/10	14/10		5	1	
	1 323/199	170/164		7	1	
1970						
6	360/24	5/33	0/25	15	<1	0
10	88/10	9/10	4/4	9	<1	1
15	179/13	500/19	2/31	14	26	<1
17	24/5	4/5	0/0	5	<1	0
19	291/19	374/19	2/2	15	20	1
	942/71	892/86	8/62	13	10	<1
Total	2 265/270	1 062/250		8	4	

[1]Emnet shows buds emasculated and bagged with mosquito netting.
[2]Untreated.
[3]Emasculated.

treatment, which was the placing of a mosquito netting bag over emasculated buds. The latter eliminated all but the smallest insects and may also have been some slight hindrance to the movement of pollen from outside.

Results

Figures from the three tests from 1966 to 1968 are combined in the top half of Table 11. For the five main clones and two others, there were seven seeds per capsule from the untreated flowers compared with one per capsule after emasculation. In the 1970 test, which dealt with the five main clones only, the corresponding figures were 13 and 10 seeds per capsule respectively. The latter high figure resulted from some exceptionally high seed yields which occurred in clones G15 and G19.

Seed yields were much less after emasculation in ten out of the twelve cases recorded separately in the table and this confirms a statement made by Pryor (1957) that emasculation stops most pollination.

Two observations were made which give a possible reason for the reduced seed set after emasculation. No bees were seen on the emasculated flowers, except briefly, and as noted on page 62 , an actual count showed that there were only a few bee visits to flowers after pollen shed was well advanced. If this explanation is correct, there is an indication here of the relative importance of bees.

Concerning the flower buds which were emasculated and covered with mosquito netting, some seed (less than one per capsule) was set. While some pollination could possibly have been done by very small insects, much of this seed could have resulted from wind-pollination or pollen falling through the net. There was support for this conclusion from the fact that seed was set after flower buds had simply been covered with an ordinary bag. This was found after bagging buds for raising selfed seedlings for grafting stocks, when seeds/capsules raised in five clones were, G6 11/10, G15 6/5, G17 2/4, G19 16/33 and G50 2/12. In this case, because of the confining effect of the bag on pollen dispersal, this simply means that some pollination can occur without insects, though not necessarily that it commonly takes place in nature.

7.2 Habits of bees and other pollinators

A statement by Grant (1950) about the limited foraging of bees was found to apply also in the seed orchard and tree bank where the area of visits has been noted

to be similarly restricted; activities are confined to one ramet over long periods, so that obviously much self-pollination occurs.

In addition, from passing observation in the tree bank, it appeared that most visits were made to flowers which had opened relatively recently and which had appreciable amounts of pollen remaining on the anthers. To test this, bees were observed on approximately 100 occasions and on a variety of clones in the tree bank from January to April 1972. If anthesis was not actually observed, the stages reached by the flowers were estimated according to the position of the stamens (p. 29). The percent visits observed were, shortly after anthesis 27%, one day after 35%, two days after anthesis 22%, three days after anthesis 12% and at later stages 4%. Thus 84% of the visits were made by two days after anthesis, by which time most of the pollen had been shed. Pollen, or pollen and nectar were evidently being collected during the first two days and mainly nectar thereafter.

From over a hundred other observations, hornets and *Lyctus* beetles were usually seen from day two onwards and taking nectar, while ants and other insects appeared at any stage, again apparently for nectar.

7.3 Pollen viability (*in vitro* tests)

Some unreplicated *in vitro* tests were done to note the percent germination after pollen storage, also after collection from flowers at various stages.

Methods

The hanging drop method (Boden, 1958) was adopted, usually with 20% sucrose solution. Preparations were examined after four hours or more, by traversing the slide systematically until 100 separately visible pollen grains had been counted.

An objective/ocular combination of 10x/6x was used when counting grains and, where necessary, 40x/6x for checking germinations. Grains and germinations were only accepted as such where identification was clear, with the pollen tube seen emerging to a length equal to or greater than the diameter of the grain. Any such tubes, including burst tubes, were counted as germinations, but groups of grains in which the individuals could not be seen clearly were disregarded.

For the purpose of testing pollen viability under various storage conditions, some fresh pollen was collected from open flowers. After mixing and placing on slides for storage, samples of pollen were collected from the slides for periodic testing.

The availability and viability of pollen were also tested at a series of flower stages, by collecting samples of pollen onto glass slides, from both bagged flowers in the field and cut flowers in the laboratory.

Results

The storage conditions tested were outdoors, indoors at room temperature and in the refrigerator with silica gel at 4 degrees C. Percentage germinations after various periods under these conditions were as follows:–

Day	0	1	2	3	4	5	6	7	11	24
Outdoors	9%	18%	3%	20%	10%	2%	1%	1%	0%	
Indoors		17%	6%	15%	14%	19%	18%	slide	spoilt	
4 degrees C.								4%	2%	3%

From the limited information obtained in this unreplicated test, a considerable proportion of the pollen could be expected to remain viable for three or four days outdoors and for at least six days indoors. No conclusions could be drawn about storage at 4 degrees C, except that some pollen was still viable up to the 24th day.

From periodic collections of pollen made from flowers as they developed, it is seen from the following statement of percentage germinations, that pollen was present on the anthers in a viable condition from day one, well past the time when the stamens had started to wither on about day four:–

Days from anthesis	1	2	3	4	6	7	8
Bagged flowers	11%	16%	1%	50%	17%	6%	
Cut flowers	22%	8%	8%	9%	11%	8%	11%

(bagged flowers, fourth day, only 30 grains available for counting)

Since the above flowers were protected in the manner stated, so as to avoid contamination with pollen from other flowers, the pollen was less subject to dispersal by insects and wind than would ordinarily be the case.

7.4 Selfing in mature flower buds

Some pollen is present on the anthers of mature buds and although pollination probably occurs when the anthers are drawn past the stigma during emasculation, very little viable seed is set (p. 24), although self-fertilised seed is produced at later flower stages (p. 66). This raises the question whether deliberate self-pollination of

Table 12

Seed yield after selfing of mature flower buds (main treatment) compared with selfing 2–6 days later (control of test 2) and with crossing mature flower buds (control of test 3)

Main bag numbers	Test/ year	Cross	Period[1]	Main treatment numbers of			Control numbers of		
				flws.	caps.	seeds	flws.	caps.	seeds
11, 77, 156,212	1/69	6x6	0	75	0	0			
282–284	..	10x10	0	10	7	8			
17, 81, 149, 294	..	15x15	0	79	65	0			
Total for test 1				164	72	8			
140, 214	2/69	6x6	0	18	0	0			
130, 200	..	..	2&3				24	4	8
5, 14	2/70	..	0	46	27	0			
2, 10	..	..	4				33	20	45
300, 361	2/69	10x10	0	35	0	0			
303,364	..	..	3				19	7	5
61	2/70	..	0	8	6	0			
57	..	..	5				27	23	53
214, 295	2/72	..	0	50	1	1			
210, 291	..	..	4&5				32	14	15
150, 376	2/69	15x15	0	21	7	0			
135, 379	..	..	2&3				24	18	1
36, 54	2/70	..	0	38	32	1			
33, 51	..	..	4&6				56	55	88
23, 48	2/70	17x17	0	42	4	0			
20,45	..	..	4&5				39	7	5
220, 315	2/69	19x19	0	44	0	0			
206, 318	..	..	3				24	4	7
29, 42	2/70	..	0	44	10	1			
26, 39	..	..	5				36	5	2
281	2/72	22x22	0	30	8	0			
276	..	..	6				24	4	0
254	2/72	45x45	0	18	15	0			
249	..	..	5				27	27	0
Total for test 2				394	110	3	365	188	229
500, 526	3/71	6x6	0	22	0	0			
504, 529	..	6x15	0				17	0	0
228, 295	3/72	10x10	0	70	5	3			
223, 306	..	10x15	0				76	24	105
509, 523	3/71	15x15	0	28	10	1			
507, 520	..	15x6	0				34	21	1
238	3/72	15x15	0	11	4	3			
233	..	15x10	0				17	5	52
193, 534	3/71	17x17	0	54	1	0			
198, 531	..	17x19	0				48	13	36
202, 539	3/71	19x19	0	17	0	0			
204, 536	..	19x17					19	8	55
281	3/72	22x22	0	30	8	0			
288	..	22x16	0				19	3	4
254	3/72	45x45	0	18	15	0			
250	..	45x24	0				49	34	67
Total for test 3				250	43	7	279	108	320

[1]Period in days from bud maturity to time of pollination.
Bags 254, 281 and 295 used both in Tests 2 and 3.

mature buds would result in any appreciable quantities of seed, or whether there is some natural barrier in operation. Some tests were done in this connection, using the five main clones and others in the tree bank.

Methods

Tests numbered 1 to 3 in Table 12 were done over a period of four seasons from 1969 to 1972. In Test 1, mature flower buds were emasculated, self-pollinated by anther and then bagged.

The same treatment (referred to in the table as the "main treatment") was repeated in Tests 2 and 3, in which concurrently done controls were also incorporated. In Test 2, for the control, the stamens used in the main treatment were collected in a match box and used to pollinate flowers of the same clone two to six days after bud maturity, the mature buds of these having been emasculated and bagged in advance. In Test 3, mature buds of the same clone as the main treatment were emasculated and cross-pollinated.

All pollens were applied fresh, at or shortly after emasculation of mature buds, and if the ages in days of the flowers from maturity to pollination are represented by the numbers 0 and 4 and the clones by the letters A and B, the pollination treatments would be represented as follows:–

Test 2	Main	0A x 0A	Test 3	Main	0A x 0A
	Control	4A x 0A		Control	0A x 0B

The entries in Table 12 consist of paired treatments, such as the above, grouped together according to the years and clones. There are many separate bag numbers recorded in the table and this is because pollinations were often done on more than one occasion during any season.

Results

The numbers of flowers shown exclude those noted as having been lost through mechanical damage, but even so, losses were very heavy, especially in the main treatments, probably due to abscission following lack of fertilisation, resulting in overestimates of numbers of seeds per capsule (p. 22).

When mature buds were emasculated and self-pollinated as in Test 1, there was a low seed yield in G10 and nil in the other two clones, the overall figure being eight seeds from 72 capsules.

When this treatment was repeated in Test 2, the yield was negligible at 3/110 seeds per capsule, compared with 229/188 when the pollen of the main treatment was used to self the more receptive stigmas of the control. Although still low, and nil in G22 and G45, the latter shows that five out of seven clones were to some extent self-fertile and that the pollen on the anthers of their mature buds was viable and available in sufficient quantity to give appreciable amounts of selfed seed.

In Test 3, after self-pollination of mature buds there were 7/43 seeds per capsule compared with 320/108 when the same clones as the main treatment were cross-pollinated at bud maturity, using the anthers of mature buds of another clone.

Since even deliberate self-pollination of mature buds yielded so few seeds, drawing of anthers over the stigma during emasculation is unlikely to result in much contamination, especially if precautions are taken. It is also unlikely that any natural self-pollination occurring within a flower as the stamens unfold a few hours later, would result in much self-fertilised seed.

Using the symbols mentioned above, the following likely reasons are given for the increase in seed yield in the controls:–

Test 2	0A x 0A	0,03 seeds per capsule	
	4A x 0A	1,2	increase due to greater stigma receptivity
Test 3	0A x 0A	0,16	
	0A x 0B	3,0	increase as barrier to selfing removed (there was also a different pollen).

The above questions of receptivity and barriers to selfing are discussed in more detail later.

7.5 Selfing at early flower stages

The emphasis here is on selfing within individual flowers, up to two days after maturity of the buds, i.e. the stage of flower development when pollen shed (p. 29) and bee visits (p. 62) present a considerable risk of self-pollination.

Methods

Mature buds were selected and some were bagged, then self-pollinated by their own anthers a few hours later, at or shortly after anthesis. The emasculation was then completed and the flowers were re-bagged.

In other cases, pollination was done at later stages and for this purpose, the stigmas were protected individually from their own pollen and were bagged. Up to

Table 13

Seed yield after selfing at early flower stages

Bag numbers	Year	Clone	Flower[1] stage	Numbers of Flowers	Capsules	Seeds
39, 40	72	G1	< 1	9	8	51
163, 164	69	G6		9	0	
108–110, 127	70			12	0	
55–57	71			10	2	0
61–63	72			6	0	
183–185, 189–190	69	G10		38	5	0
246	70			10	10	0
194, 195	69	G15		19	0	
106, 113, 114, 137	70			12	9	0
51, 52	71			9	4	0
29	72			5	3	0
173, 176, 177	69	G19		13	0	
				152	41	51
167, 168	69	G6	1	19	3	0
119, 121, 123	70			8	8	11
58, 59	71			8	0	
48–50, 61	71	G10		23	10	23
70, 71, 73, 84, 86	72			21	4	2
186, 188, 191–193	69	G15		24	0	
103, 115, 116	70			7	6	2
37, 67	71			5	0	
139, 140	70	G17		13	1	0
24	71			3	0	
172, 174	69	G19		8	0	
131, 133, 134	70			14	1	1
1, 2	71			4	0	
				157	33	39
38	72	G1	2	6	1	2
169, 170	69	G6		9	3	1
117, 118	70			7	2	0
15	71			3	0	
52–54, 57–59	72			15	3	1
180–182	69	G10		21	4	3
245	70			4	0	
69, 80, 81, 83	72			14	1	1
100–102	70	G15		10	10	2
35, 36	71			9	1	3
25, 48, 49	72			11	9	1
141, 142	70	G17		16	4	1
171, 175	69	G19		7	0	
132, 135	70			11	1	1
3, 4	71			8	0	
				151	39	16
Total				460	113	106

[1]Flower stage at which pollination was done, in days from the time of anthesis: <1 represents the first few hours after anthesis.

two days later, the protection was removed from the stigmas and each flower was pollinated with its own anthers, followed by removal of all remaining stamens and re-bagging. The protection of individual stigmas was done by covering each with the tip of the operculum, the base of which had been cut away to allow unfolding of the stamens. The tip of the operculum was held in place by narrow strips of adhesive tape. The process was continued over four seasons from 1969 to 1972 in the tree bank.

Results

As shown in Table 13, flower losses were very heavy due, it is believed, to handling during protection of the stigma from its own pollen, as well as to abscission following lack of fertilisation and it was hardly feasible to obtain sufficient capsules for reliable results.

Under the heading "flower stage", the entry <1 represents the first few hours after anthesis. At this stage, excluding G1, there were no seeds from 33 capsules in 4 of the five main clones and this conforms with the result from pollination at an earlier stage in the previous section. On the first and second days after anthesis there were 1,2 and 0,4 seeds per capsule respectively, so that the risk of self-fertilisation within flowers of the five main clones was quite small during the first two days, especially in comparison with the apparent risk of self-pollination. During this period, neither pollination resulting from pollen shed within a flower, nor from insect visits to individual flowers, would be likely to yield much selfed seed in these clones.

By contrast, in G1, there were 6 to 7 seeds per capsule from pollination shortly after anthesis. Therefore although selfed seed was rarely produced after pollination of most clones at early flower stages, exceptions are to be expected.

7.6 Receptivity

Receptivity was assessed on two bases, according to seed yield after pollination at various flower stages, and according to germination of pollen on the stigma at these stages.

Receptivity based on seed yield

Tests were done with the object of observing the onset and duration of receptivity and the flower stage when pollination gives the greatest seed yield.

Methods. Pollination (mainly cross-pollination), was started in the tree bank and was extended to the seed orchard, the tests being numbered serially from 1 in 1966 to 14 in 1968 (number 11 is missing because the tree was blown down).

In each test, mature buds were emasculated and bagged daily, or as frequently as possible, for periods of eight days or more. A few buds were also included which had not quite reached the mature stage. Flowers of a series of ages were thus made available by the end of the period, at which time they were all pollinated on one day. Notes were also made on the appearance of the stigmas in terms of shape, colour and the condition of the surface, as described on page 29, after which the flowers were re-bagged.

Pollen was applied "by anther" in Tests 1 to 9, but "by brush" for the remainder. An attempt was made to cover a wide range of possibilities by doing a variety of single crosses. In Test 1, self-pollination as well as cross-pollination was done, but only the latter in subsequent tests.

The procedure of preparing flowers in advance for pollination on one day was adopted in order to reduce the effect of day to day differences in pollinating conditions. This also permitted the use of the same pollen source throughout any one test, without having recourse to the use of stored pollen. While confining emasculation mainly to mature buds no doubt gave flowers at the desired stages of maturity within reasonable limits, the procedure nevertheless imposed a limitation on the number of buds which could be emasculated on any one day. This, combined with losses of capsules, resulted in some undesirable gaps in the daily records, also in fewer capsules per treatment than would have been desirable in some cases.

Details of each test are given in Appendix 1 and the results are summarised in Table 14, where the most distinctive stigma characteristics, the swollen outline and sticky surface are shown by upper and lower horizontally drawn lines under the appropriate days. For the sake of clarity, other features of the stigma such as colour and pointed or rounded shape, which are less distinctive, are not represented.

Results. To test the earliest age at which cross-pollination can result in seed, some of the buds shown in Table 14 as having been pollinated at anthesis, were actually pollinated an estimated one to two days before that stage and, as may be seen from the last footnote to Appendix 1, seed was even produced from buds at this early stage.

Table 14

Stigma receptivity on the basis of numbers of seeds per capsule after pollination of flowers for the first ten days after anthesis, in the tree bank and in the seed orchard

Test & cross	Number of seeds per capsule when pollinated after 0	1	2	3	4	5	6	7	8	9 & 10 days	Mean Nr. of capsules used
3/67 6x17	3	6	6	11	0	36	12				6
7/68 6x19	1	2	1		16	37	13	24	0		9
1/66 15x15		<1	7	7	4		<1		0		9
1/66 15x17		2	22	34			12	9	<1		9
8/68 15x19	26	2	15	22	24	32	32	12	13		16
2/67 17x6	5		<1	<1	16	15	1				4
9/68 17x19	10	9	15	16	22	27	11	20	9		10
10/68 19x15	3	2	28	40	44	48	33	7	19		10
4/67 10x17		1	18				40				10
13/68 10x6	3	10	11	31	9	33	42	48	44	42 45	11
5/67 15x17		<1	3		11				25		8
14/68 15x6	2	2	1	18	36	41	59	58	46	45 35	18
6/67 19x17	5	4	4		24		37		20		6
12/68 19x15	14	25	31	44	42	45	29	12	12	37	7

The pairs of ruled lines below the typed figures show:—
upper line, the flower stage when stigmas were swollen.
lower line, ... sticky.

Blank spaces within the series of flower stages show either that no capsules survived, or that no pollination was done.

The top half of the table shows results of pollinations in the tree bank, the lower half in the seed orchard.

Most buds pollinated at anthesis yielded some seed and it therefore appeared, on the basis of seed yield, that the stigmas were receptive at this stage and, in the tree bank at least, even before it. The stigmas were green and pointed at this time and did not present a receptive appearance compared with later stages.

Concerning the end of the receptive period, it is evident that there is some variation according to site and no doubt also depending on the particular clone. In Test 7 of G6 in the tree bank, judging from the nil result after pollination on the eighth day after anthesis, receptivity ended on the seventh day. Three of the other crosses at this site still showed appreciable though decreasing yields on the eighth day and it is possible that the condition could have lasted a few more days in these cases, or several more where styles were seen to persist for long periods (p. 30).

In the seed orchard, the receptive period was longer, since seed yields were still high on the eighth day and in two cases, even on the tenth day.

In connection with the self-pollination of G15 in Test 1, there was only a limited period, from the second to the fourth or fifth day when pollination resulted in an appreciable seed yield.

Heavy capsule losses attributable to abscission were seen in several tests. As recorded in Appendix 1, these were mostly outside the main receptive period. Although the numbers of seeds per capsule are liable to be over-estimates in these cases (p. 22), this does not affect the conclusions which follow.

In six tests of four clones in the tree bank, the daily records are sufficiently complete to show that seed yield was highest after pollination done on about the fifth day after anthesis. In three clones in the seed orchard a comparable stage was reached on the fifth to the seventh day after anthesis, so that depending on the cross concerned, there was more variation here and a tendency to be later. The cross G19x15 was tested in both sites and the maximum occurred on the fifth day in each case.

The swelling of the stigma corresponded fairly closely with the above maxima, irrespective of site, therefore this feature could be used as a guide to the best time for pollination. This would be more or less when the stigmas begin to swell, but if this condition and the formation of a sticky appearance became advanced, this would indicate a decline in receptivity, especially in the tree bank.

Receptivity based on pollen germination

The intention was to observe receptivity on the basis of germination of pollen on the stigma at various flower stages, as done by Gabriel (1966).

Methods. Trials were made of several ways of preparing slides of control-pollinated stigmas for microscopic examination. These included the preparation of paraffin embedded sections, the use of fresh material for sectioning by hand and with a freezing microtome, also smearing as described by Datta & Naug (1967).

During these trials, as in the case reported by Gerstel & Riner (1950), it was found that when stigmas were examined which had been pollinated at an early stage, grains were lost during transfer from one reagent to another, hence a process with the minimum number of such transfers was required and it would appear that water soluble waxes (Sass, 1966), or the carbowax embedding technique (Hibben, 1968) might be suitable. However, since these materials could not be obtained at the time, a trial was made of sectioning freshly cut stigmas under a large drop of xylol on a slide. After pollination therefore, styles were placed directly into the xylol and stigmas were sectioned longitudinally by hand while being held in position on the slide, or the pollen grains were deposited into the xylol simply by scraping the stigmas with a needle. This involved actually passing the needle through the stigmatic tissue, in the course of which some of the stigma cells were removed into the xylol. After partial evaporation of the xylol, slides were prepared with D P X mountant.

Slides were examined in a manner similar to that described on page 62, but in cases where there were large numbers of ungerminated grains, the counting of these was approximate only, in that the numbers were estimated in groups of five or ten. In respect of germinations also, the numbers were in some cases approximate, as in the case of grains and tubes obscured by stigmatic tissue.

The shortest time for commencement of germination was noted during preliminary trials to be four hours, and in the case of fully receptive stigmas about eight hours was at first used as a suitable period from pollination to sectioning. Later, germinations under some conditions were found to have only just started after this period and as a result, it was convenient to leave stigmas overnight, involving periods of up to 15 hours. It was found desirable to avoid periods much longer than this, because the grains collapsed and became difficult to identify, also the tubes became attenuated and less easy to observe.

In regard to the controlled pollination procedure, five separate crosses were done at the tree bank in 1969 and 1970. As shown in Appendix 2, these were G6x9, G10x17, G15x(1+9), G17x(3+9) and G19x(3+9).

Table 15

Stigma receptivity on the basis of numbers of pollen grains, germinated and ungerminated, on stigmas pollinated during the first four days after anthesis

Days from anthesis to pollination	<1[1]	1	2	3	4
Numbers of stigmas examined	17	22	17	14	12
Numbers of stigmas with germinating pollen grains	2	1	5	13	11
Numbers of ungerminated pollen grains (hundreds)	48	78	92	134	142
Numbers of germinated pollen grains	2	1	205	691	700
% stigmas with germinations	12	5	29	93	92
% germination of grains	< 0,1	< 0,1	2	5	5
Numbers of germinations per stigma.	0,1	< 0,1	12	49	58
Numbers of ungerminated grains per stigma	282	355	541	957	1 183

[1]Pollinations done during the first 12 hours after anthesis.

Table 16

Earliest recorded germination of pollen on the stigma

Serial Nr.	Cross	Number stigmas examined	Germinations nr.	Germinations days[1]	Period pollination to sectioning
7	10x19	4	1	0	5 hours
13	17x19	2	1	0	6 ..
16	17x19	2	1	0	20 ..
41	19xmix[2]	2	1	0	21 ..
92	19x(10+15)	4	3	1	17 ..

[1]Period in days from anthesis to pollination.

[2]Cross-pollination with a mixture of pollens from several clones.

Mature buds were emasculated and bagged daily in advance as described on page 69. Pollination was done by brush and in each of the five crosses, this was done on one day using the same pollen source throughout, followed by re-bagging. Pollination of cut flowers in the laboratory was tried, but was discontinued in favour of pollination under more natural conditions on the tree.

Results. Results for the five main clones recorded in Appendix 2 are summarised in Table 15, where the numbers of ungerminated grains are rounded off to the nearest 100. This table shows the numbers of germinations of pollen grains up to the first 17 hours after pollination and not the result of accumulated germination over the life of the stigma, as in the previous section.

Some germinations occurred on each day, but the three at or near anthesis are regarded as isolated cases, probably of an *in vitro* nature and not followed by penetration of the style. Two days after anthesis there were 12 germinations per stigma, but as may be seen from the appendix, these occurred only in clones G6 and G19 and not in the other three clones, though as from the third day, all clones had some germinating grains. If these and the other results in the table reflect the onset of receptivity, this started two to three days after anthesis, rather than at anthesis, as appeared to be the case on the basis of seed yield. To distinguish between these two assessments of receptivity, they are referred to as "true" and "effective" receptivity respectively, pollination in the former case being assumed to be followed without delay by tube growth.

There was an increase to 49 and 58 germinations per stigma after pollination done three and four days respectively from anthesis, and unrecorded slides at later stages showed further increases. These figures, also the percent germination of grains and the percent stigmas with germinations, shown in the table, apparently reflect a gradual increase in receptivity and/or an increase in the number of stigmas which have developed a receptive condition.

There was a regular increase in the number of ungerminated pollen grains remaining on the stigma, corresponding with increasing stigma age, from 282 per stigma after pollination during the first 12 hours after anthesis to 1183 after pollination on the fourth day. Thus, assuming that stigmas in each treatment received comparable amounts of pollen, during the short period from pollination to sectioning, the less advanced stigmas lost a higher proportion of grains than was the case with stigmas at later stages of development.

Table 17

Number of pollen grains, germinated and ungerminated, on stigmas pollinated at anthesis and left for periods of up to 5 days before sectioning, compared with the same data from stigmas pollinated at maximum receptivity

Days from anthesis to pollination	0	0	0	0	0	4 to 5
Period from pollination to sectioning.	1 day	2 days	3 days	4 days	5 days	6–9 hours
Number of stigmas examined	10	8	10	5	6	7
Number of stigmas with germinating grains.	0	0	0	3	5	7
Number of ungerminated grains (hundreds)	13	4	7	6	4	30
Number of germinated grains	0	0	0	15	11	166
%stigmas with germinations.				60	83	100
% germination of grains.				3	3	6
Number of germinations per stigma.				3	2	24
Number of ungerminated grains per stigma	130	50	70	120	67	429

As regards the above mentioned occasional germinations at early flower stages, others which were noted during the preliminary trials are recorded in Table 16 which supplies extra evidence of occasional germinations on stigmas at or shortly after anthesis.

7.7 Behaviour of pollen on stigmas at anthesis

It is clear from page 63 that pollen is capable of retaining its viability so that it can germinate several days after it has been placed on a stigma, also that considering the last two sections combined, this is indeed what occurs and that after pollination of a stigma at an early stage, there is typically a resting period followed by germination and (presumably) penetration of the style. It is nevertheless of interest to demonstrate this more directly.

Methods

Three separate crosses were done at the tree bank in 1970, namely G6x50, G17x15 and G19x15, as shown in Appendix 3.

Buds at or approaching maturity were emasculated, cross-pollinated by brush and bagged on one day, followed by daily sectioning up to five days later. As a control, the same pollen sources were used to pollinate flowers which had been emasculated at maturity four to five days earlier. For processing and examination of the stigmas, the same procedures as those given in the previous section were used.

Results

The figures in Appendix 3 are summarised in Table 17 where the numbers of ungerminated grains are rounded off to the nearest 100.

The pollen which was placed on stigmas at (or approaching) anthesis did not begin to germinate until four days later. This delay, compared with the prompt germination after six to nine hours on receptive stigmas (four to five days after anthesis), is attributed to lack of receptivity, i.e. true receptivity (p. 74). If this result is considered in conjunction with the pollen losses on young stigmas seen in Table 15 (p. 73), it is clear that this loss would represent one of the causes of low seed yield after controlled pollination at early flower stages.

It is noted that this result would indicate an amendment of the previous assessment of the onset of true receptivity, except that treatments were different, especially in that some buds were pollinated before anthesis.

7.8 Summary and conclusions

The changes that take place in the development of the flower are summarised in Table 18, which includes some observations from Chapter 5, as well as the present chapter.

In tests of stigma receptivity, seed was produced from flowers cross-pollinated at and even before anthesis, so that it appeared that a receptive condition had already developed by that time. Maximum yield was obtained after pollination on the fifth to the seventh day when the stigmas were becoming swollen; thereafter a declining receptivity was observed. There were distinct day to day changes in yield and stigmas remained receptive to cross pollen in varying degrees up to the seventh day after anthesis or later. From a single test by self-pollination, not only was the yield of seed per capsule less than that after crossing, but the period during which pollination resulted in seed production was also less, being from the second to the fourth or fifth day from anthesis.

Receptivity was also tested according to germination of pollen on the stigma, but in this case, although occasional germinations were observed at anthesis, these were probably of an *in vitro* nature and the main germinations commenced two or more days later, depending on the clone and on the treatment applied. Hence stigmas should not be regarded as receptive at anthesis, as appeared to be the case from results of tests based on seed yield.

The results of germination of pollen on the stigma are referred to in Table 18 as indicating "true receptivity", compared with the results according to seed yield which are recorded as indicating "effective receptivity". In the latter, seed yield after pollination at anthesis was shown to occur as a result of pollen remaining on the stigma in a viable condition for two or more days before germinating.

In any one flower, the end of the main pollen shed coincides with the beginning of true receptivity (to cross pollen) in some clones, and this is taken to indicate a protandrous condition which, however, is partial rather than absolute.

It was found that wind, or the dropping of pollen, could result in some fertilisation, but the amounts of seed set were small and there was confirmation of the commonly held view that the flowers are mainly insect pollinated. Many types of insects were observed visiting the flowers and of these, the honey bee seemed to be the most effective pollinator. This is because of its active habits and because bees were rarely seen on emasculated flowers which, in turn, usually gave much re-

Table 18

Stamen unfolding, pollen shed, receptivity and insect visits at various flower stages

Days after anthesis			0	1	3	5	7
1	Anthers pass stigma		XXX				
2	Stamens fully extended			XX			
3	Stamens withering	dry				XXXXXXXXX	
		wet			XXX		
4	Pollen shed (dry)		x x x XXXXXXXXXXX x x x				
5	Pollen viable		XXXXXXXXXXXXXXXXXXXXXXXXXXX				
6	. . retained on stigma		x	x	x	xxxXXX	
7	True receptivity				x x x x	XXXXXXXXXXXX	
8	Effective . .		x x x x x x x x x	x	xxxxxxxxXXXXXXXXXXXXXXXXX		
9	Selfed seed produced		x	x	x x x	xxXXXx	
10	Receptive appearance					x x x	XXXXXXXXXXX
11	Nectar visible			x x. x x x x x x	x	x	x
12	Bee visits		XXXXXXXXXXxxx x x x x		x	x	x
13	Other insects		x x x x x x x x x x x		XXXXXXXXXXX		

x x x ———— shows an attribute in a slight degree.

xxx ———— shows an attribute in a greater degree.

XXX ———— shows an attribute at maximum.

Item 3, "dry" and "wet" refer to weather conditions.

Item 7, receptivity assessed according to germination of pollen on the stigma.

Item 8, receptivity assessed according to seed yield.

duced seed yields. Bees were also rarely seen on flowers after the main pollen shed, but the other insects differed in that visits were made at a variety of flower stages. Another important aspect of the habit of the honey bee is its persistence in foraging on one ramet for long periods.

Seed yield after self-pollination of mature flower buds was usually negligible, but the yield was 40 times greater when the same pollen was used on flowers about four days after anthesis; similarly yields were 18 times greater when cross pollen (instead of self pollen) was applied at anthesis. These increases were attributed to greater receptivity as the flowers develop and, in the second case, to removal of some barrier to selfing. These tests also served to show that the clones concerned were self-fertile and that there was sufficient pollen on the anthers of mature buds to give rise to appreciable amounts of seed.

The finding that flowers were not easily self-fertilised as a result of pollination done on mature flower buds also applied throughout the next two days, when the shedding of pollen and activity of bees lead to the likelihood of much self-pollination within individual flowers. With one exception, seed yields were low at about one per capsule or less during this period and the risk of selfing was small.

It therefore appeared that an unspecified barrier was in operation during the first two days after anthesis, which prevented most intra floral selfing. In nature, however, there remains the possibility of selfing between flowers at various stages. For example, if insects were to transfer pollen from a newly opened flower to another which had been open for some three or four days, much self-fertilisation would be liable to result. But since bees rarely visit flowers which have been open for more than two days, the amount of self-pollination resulting in self-fertilisation by this agency would be more limited than might be supposed. However, this would only apply in the case of bees, as other insects were seen on flowers at various stages.

Since pollen retained its viability outdoors for about four days, it is assumed that little or no part would be played in controlling selfing by loss of pollen viability while it remained ungerminated on the stigma. But when pollen was placed on stigmas at gradually increasing stages of receptivity and a count was made up to 17 hours later, there was a regular increase in the number of grains which were retained, from 282 per stigma pollinated at or near anthesis, to 1183 per stigma pollinated four days later. This pollen loss from as yet unreceptive stigmas, com-

bined with a considerable degree of protandry, apparently controls the amount of pollen remaining on the stigma for possible germination later.

Reverting to the effects of the habits of insects, if foraging by the main pollinators is mostly confined to the first two days after anthesis, as well as limiting selfing, this would also limit the amount of cross-pollination in nature and could account for the fact that seed yield after a single application of cross pollen is usually much greater than that after open-pollination (Table 20, p. 87).

Chapter 8

BREEDING SYSTEM

An outline is given on pages 9 to 15 of the factors which control the relative amounts of selfing or crossing in nature, the breeding system found in trees, inbreeding depression, experimental procedures used in determining natural selfing, and barriers to selfing.

The aspect of reproductive behaviour in plants which is most applicable to this chapter is the occurrence of selfing and its effect. Of the three main breeding systems, the concern is with inbreeding and outbreeding, no study having been made of any form of apomyxis (Stebbins, 1941).

8.1 Tests done with *E. grandis*

In a series of tests done from 1967 to 1972, mainly in the tree bank, the subjects investigated are those listed below:–

(a) relative yield of seed after self-pollination compared with cross-pollination.

(b) incidence of self-fertility among clones,

(c) inbreeding effects,

(d) natural selfing and

(e) barriers to selfing.

Certain details of treatments applied in dealing with these subjects (except b) are given in Table 19, where the various tests are numbered serially.

Under the heading "application of pollen", a considerable variety of treatments is shown in the table and the choice of these depends not only on the objects of the tests, but also on the numbers of flowers available and the clones in flower at the time, their characteristics and the state of knowledge about them when each test was done. In the next three paragraphs, an explanation is given of these various treatments.

Early practice had been to apply pollen "by anther" and although this was continued for a while, pollination "by brush" was then introduced, mainly so that mixed pollens could be applied. The procedure for the collection, mixing and application of mixed pollens is given on page 22.

Table 19

List of tests done on the breeding system, main subjects, pollination treatments and methods of pollen application

Test number and year	Main[1] subjects	Pollination[2] treatments	Application of pollen
1 to 8[3] 1967 & 1968	a&c	S&X	By anther, to the five main clones and to flowers at comparable stages.
9 to 12 1968 & 1969	a&c (e)	S, X&m	By brush, to the five main clones and to flowers at comparable stages.
13A to 17A 1970	a&c (e)	S, X&M	As above.
13B, 14B, 16B, 17B	c (e)	S, X&M	As above but to flowers at chosen stages.
15B 1970	c, d&e	S,X&M	As above.
18 1971	c(d&e)	S,X&M	By brush, to a selected clone with suspected deviant progeny and to flowers at various stages.
19 1971	c&d	S&5X	S by anther and 5X by brush, to a selected clone with deviant progeny and repeatedly as receptivity developed.
20, 21 & 24 1972	a, c, d&e	S,5X&M	By brush, to selected clones with deviant progeny and to flowers at comparable stages.
22 1972	(a, c, d&e)	S,5X&M	As above.
23 1972	a&c (d&e)	S,5X&M	As above.
25 & 26 1972	a&c (d)	S&5X	As above.
27 1972	d	S	By anther, to a selected clone and repeatedly as receptivity developed.

1 Tests intended for assessment of relative yield, a; of inbreeding effects, c; of natural selfing, d and of barriers to selfing, e. (Tests of the incidence of self-fertility among clones "b", not being numbered serially as are the other tests, are not recorded in the table); a & c (e) shows that the tests were intended for barriers to selfing (e) but they were mainly failures for this purpose, although results were used for "a" relative yield and "c" inbreeding effects.

[2]S, self-pollination; X, cross-pollination using pollen from a single clone;
5X, cross-pollination using pollen from five different clones mixed in approximately equal proportions;
m, mixed-pollination using the self and cross pollens mixed in approximately equal proportions and
M, mixed-pollination using the self and cross pollens mixed in equal proportions by weight.

For the 5X pollination treatment, the clones used were:–
Test 19, G17, 19, 38, 58 and 101, and for Tests 20 to 26, G16, 17, 19, 47 and 101.

[3]Test 8, though not originally intended for estimating natural selfing was eventually found suitable for that purpose.

Though not indicated under treatments, capsules from open-pollination were also used.

Tests numbered 3,4 and 9 to 11 were pollinated in the seed orchard, all others in the tree bank.

Tests numbered 6 and 7, 9 and 10, 13 and 14, 15 and 16 were done in reciprocal pairs (p. 84).

The five main clones used at first were among the earliest to come into production and they were accepted for use even though nothing was known about their suitability for various types of tests. As more clones became available they were used in various tests after observations had been made as to their suitability in regard to fruit set (p. 42), and after they had been tested for the production of deviant progeny for use as markers in estimating natural selfing.

Flowers were taken for pollination at "comparable stages" in all tests of relative yield, in order to limit the effect on seed yield of changes in receptivity at various flower stages. This included tests 13A to 17A which were done concurrently with and using the same pollen source as that used for tests 13B to 17B. In the latter, flowers were pollinated at "chosen stages" in those tests of selective fertilisation in which an attempt was made to eliminate the effect of varying numbers of seeds per capsule on early vigour (p. 113). The simpler procedures of pollination "at various stages" or "repeatedly as receptivity developed" were applied in tests where results were not based on yield of seed or on early vigour. The latter procedure of repeated pollination was adopted when flowers were scarce and it was necessary to make use of all that were available.

Tests numbered 1 to 8 were exploratory in nature, in that they involved the application of single pollens, rather than the mixed pollens which were applied in later tests.

In Tests 9 to 12, self and cross pollens were mixed in approximately equal proportions, but in all subsequent tests they were mixed in equal proportions by weight.

Tests 9 to 17 were primarily intended for an investigation of selective fertilisation, but most of them were later considered unsuitable for that purpose (p. 110), although some were used for tests of relative yield.

8.2 Relative yield

This refers to the yield of seed after self-pollination compared with cross-pollination, as investigated by Bingham & Squillace (1955) and others, for pines and other genera.

Methods

Controlled self- and cross-pollinations were done and the relative seed yields were calculated from the number of germinations per capsule (selfed) over the number of germinations per capsule (crossed), times 100.

Although not part of the relative yield assessment, it is convenient to include here the methods used in measuring epicotyl length, germination time, heights and bole straightness for the purposes of subjects (c), (d) and (e) of Table 19.

For assessing relative yields, many pollinations were done in the form of "main tests", supported by a "pollen test" as below. When feasible, two main tests were done in reciprocal pairs, so that a single pollen test could serve for both. The crossing pattern then was:—

	Main test		Pollen test	
	self	cross	cross	cross
Test 1	AxA	AxB	CxA	CxB
Test 2	BxB	BxA		

In preparing for controlled pollination, the first step was to find flower buds at comparable stages of development, the "mature stage" being used (p. iv). Mature buds were therefore emasculated and bagged, usually on one day, then three to five days later, after buds had been bagged for a pollen source, treatments were allocated randomly to inflorescences and the flowers were pollinated on one day and re-bagged. An exception to this was in Tests 1 to 3 and Test 6 where, in order to find as many mature buds as possible, comparable numbers of these in each treatment were emasculated on two consecutive days, so that there were two flower stages at pollination which differed by one day.

After reaping, the contents of each capsule were extracted and placed separately in the Jacobsen apparatus and the numbers of germinations were counted.

Where required, the progeny were also measured for germination time, epicotyl length in the nursery and total height after planting.

To note the dates when each germination occurred, the pads of the germinator were examined daily (except Sundays) during the first eight days when germination was at its peak, then daily except Saturdays and Sundays. The mean germination time was calculated from the number of daily germinations (f) and the number of days taken to germinate in each case (x) using the formula $\Sigma (fx)/\Sigma f$.

In preparation for the measurement of epicotyl length and eventual planting, the germinating seeds were transferred individually to polythene tubes as described on page 25, except that in Tests 3 to 11, seeds were allowed to remain on the germinating pads for about two days before transferring, so as to observe germination. In all tests except subject b, allocation of germinating seeds to tubes was done

randomly. In Tests 1 to 17, each seedling was labelled with a serially numbered ticket, thus making it possible to relate individual seedlings to the capsules from which they were derived. These data were used in connection with assessments of selective fertilisation (p. 110).

As there was often marked inequality between treatments in numbers of seeds, especially in that there were usually too many crosses, it soon became the practice to emasculate fewer buds for crossing than for selfing (and intermediate numbers for mixed pollination). But where this procedure still failed to achieve the object, capsules or seedlings were randomly discarded until there were comparable numbers in the treatments.

To measure epicotyl length, after the growth of the hypocotyl had ceased, the level of insertion of the cotyledons was marked by a wire made into the shape of an inverted U and pushed into the soil. Measurements were taken of surviving seedlings to the nearest mm, from the top edge of the wire to the axils of the highest clearly separated leaf pair.

Several of the numbered tests were planted and for this purpose seedlings were taken randomly and planted in randomised blocks with line plots at an espacement of 3x3 m, with a single row surround to each complete test. In tests of natural selfing (except for Test 8), seedlings not taken randomly were planted unreplicated outside the formal lay-out.

After planting, heights were measured by height rod, at 11 to 18 months after sowing the seed, the intention being that this measurement should be taken so as to give as much time as possible for inbreeding effects to appear, but to measure before competition set in. If a transplant had been classed as abnormal (p. 51) but was dead by the time measurement for height was done, it was assumed that this was a treatment effect and the tree was allocated a zero value for height. Otherwise, when there were clearly other causes of death, such as attack of the roots by termites, the tree and its height were disregarded. Dead plants were replaced where feasible, but such replacements were not included in the measurements.

Some observations were also made at a later stage after competition had set in, to observe bole straightness and vigour as represented by relative dominance.

The bole of each tree up to about three quarter height was inspected from all directions by two observers and note was taken mainly of "crook" or sharp devi-

ations from the straight, also "sweep" or extended curves, these two being referred to below as defects. Less account was taken of sweep as this is probably largely an environmental effect.

A subjective point scoring system was used for straightness and an assessment of relative dominance was done at the same time, the allocation of points being as follows:–

straightness	points	relative dominance	points
No appreciable defect	10	dominant	10
1 slight defect	8	3/4 height of ..	7
1 clear or 2 slight defects	6	1/2	4
2 clear or >2 slight defects	4	1/4	2
>2 clear defects	2	dead & dying	0
no appreciable length of straight stem	0		

Intermediate points for straightness were allocated where appropriate.

Trees which began to develop abnormalities after planting and which appeared likely to die early, were watered and shaded as necessary to keep them alive long enough to identify the type of abnormality. They were then classified on the basis of the notes on this subject on pages 51 to 56 . In assessing normal to deviant ratios, account was taken only of those trees which survived long enough to develop the type of defect under observation in each case.

Regarding the calculation of relative yield, in those cases where a pollen test had been included (p. 84), if a Chi-square test indicated a significant difference at the 1% probability level in the numbers of germinations per capsule from the two pollens, then an adjusted relative yield was calculated in proportion to the numbers of seeds per capsule from the two pollens in the pollen test. This was done on the assumption that a more realistic estimate of relative yield would thereby be obtained.

Results

The results in Appendix 4 give details of the calculation of relative yields, including the pollen tests and the resulting factors which were used in calculating adjusted relative yields. Numbers of flowers pollinated and capsules reaped are also shown, omitting losses noted as being due to mechanical damage. It is assumed that the remaining losses shown were due to abscission and that this was associated with lack of fertilisation (p. 22). Where such abscissions were appreciably greater after selfing than after crossing, the calculated number of selfed seeds per capsule should

Table 20

Numbers of capsules, seeds per capsule and relative yields of seed, after self-pollination compared with cross-pollination (M mixed-pollination and 0 open-pollination are included here for convenience only)

Test[1]	Year	Clone	Numbers of capsules/seeds per capsule				Relative yield	Cross pollen
			M	O	S	X[2]	S/X	
1	'67	G6		7/15,9	7/0,9	7/27,1	3	G19
5	'68			18/7,2	21/6,6	9/22,0	30	..
12	'69			17/4,9	45/1,2	30/4,6	26	..
13A	'70		7/0		11/0	14/0,4		..
				42/7,7	84/2,4	60/8,9	27	
3	'67	G10		10/5,7	22/8,1	13/48,5	17	G17
9	'68			23/2,3	38/0,9	17/67,6	1*	G15
17A	'70		34/3,8		20/0,5	24/12,2	4	G17
				33/3,4	80/2,8	54/38,4	7	
8	'68	G15		25/2,6	32/3,8	20/46,3	8	G17
10	'68			30/3,1	30/3,7	14/55,6	7	G10
15A	'70		29/33,2		24/10,3	22/53,0	19	G17
				55/2,9	86/5,5	56/51,3	11	
4	'67	G17		6/1,8	5/0,9	5/31,8	3*	G10
7	'68			3/14,0	16/0,4	28/20,4	2*	G19
11	'68			24/4,9	23/0,8	18/41,8	2*	G6
16A	'70		28/22,7		32/0,9	22/37,7	2	G15
				33/5,2	76/0,8	73/31,7	3	
2	'67	G19		19/2,9	6/0,07	11/3,5	2	G6
6	'68			10/8,4	39/1,8	30/45,7	4	G17
14A	'70		19/8,9		18/1,0	19/32,7	3*	G6
				29/4,8	63/1,4	60/33,9	4	
			M	O	S	5X[3]		
24	'72	G13	21/28,4	21/8,1	42/26,2	11/56,0	47	
25	'72	G34		12/15,8	18/9,9	6/28,5	35	
21	'72	G30	15/23,3	16/8,6	17/10,1	10/31,1	32	
20	'72	G1	15/20,0	50/4,4	24/8,7	8/29,4	30	
26	'72	G9		8/5,1	17/3,9	6/20,5	19	
23	'72	G24	8/19,4	2/25,5	16/1,8	6/24,2	7	

[1]The numbered tests refer to those listed in Table 19, page 82 .

[2]S, self-pollination; X, cross-pollination using pollen from a single clone; M, mixed-pollination using the self and cross pollens mixed in equal proportions by weight and O, open-pollination.

[3]In the lower part of the table, 5X indicates cross-pollination using pollen from five different clones mixed in approximately equal proportions.

*These relative yields are likely to be over-estimates (p. 88).

be fewer than that stated and hence, the recorded relative yield is likely to be an over estimate. This is indicated by marking the figures concerned in Table 20 with an asterisk.

The figures in Appendix 4 are summarised in Table 20, where mixed- and open-pollinations are included, although these are not used in the present context. The bases in terms of numbers of capsules and seeds per capsule are shown, as well as the relative yields.

The early tests numbered 1 to 17, in which single cross pollens were applied are shown in the top part of the table, and are considered first.

G6, in which the same pollen was used in four consecutive seasons, showed a very big range in relative yield, from nil to 30%. It is believed that this is associated with the observation that the stigmas of this clone sometimes fail to develop a receptive appearance (p. 30). (The results in Table 7, page 41, where only 55% of the capsules had seed after cross-pollination compared with much higher figures in other clones, appears to be connected with the same feature).

The figures for G10 varied from 1% to 17% and those for G15 from 7% to 19%.The results for these two clones therefore covered a somewhat similar range and were comparable when their mean values are considered.

In the various tests of G17 and G19 conducted over a series of seasons, relative yields were consistently very low, irrespective of the cross pollens used and the figures, which varied from 2% to 4%, were probably over estimates in some cases.

The mean figures for these clones were, G6 27%, G10 7%, G15 11%, G17 3% and G19 4%.

Later tests, in which mixed cross pollens were applied, are recorded in the lower part of the table, where they are arranged in descending order of relative yield.

Test 22 of G15 failed for the present purpose, because all the cross-pollinated capsules were lost through mechanical damage and for this reason it is omitted from the table.

Most of the pollen tests also failed in this series, and that for Test 21 was of doubtful value because there were only three self and four cross capsules in the pollen test and its application would have given a far higher yield from selfing than from crossing. Although this is not impossible, it was considered that the unadjusted figure shown in Appendix 4 would be more likely to be representative.

The first four clones listed (G1, 13, 30 and 34) had relative yields of 30% to 47%, which is much higher than the clones previously tested and these clones would be considered for rejection for breeding purposes, especially if many deviant types were produced among their progeny. The remaining two clones, G9 and G24 were comparable with G10 and G15, with relative yields of 19% and 7% respectively.

Thus, if one test of G6 is ignored, the eleven clones tested showed a wide range in this feature, from a negligible 2% to as much as 47%. There is also an indication of seasonal variation in the relative yields of G10 and G15, though not in that of G19:–

G10x17	1967	relative yield	17%
. .	1970	. . . .	4%
G15x17	1968	. . . .	8%
. .	1970	. . . .	19%
G19x6	1967	. . . .	2%
. .	1970	. . . .	3%

8.3 Incidence of self-fertility among clones

Having found that each of the five main clones initially used in the previous section were in varying degrees self-fertile, as more clones were accepted and came into production, it became possible to broaden the scope of observations on the incidence of self-fertility among individuals. To increase this still further, it was decided to include not only accepted clones, but also those which, although they had been retained in the tree bank, had been rejected for breeding purposes.

Seedlings were raised in this connection and these served for the purpose of observing abnormalities (Table 10, p. 54), and as preliminary tests in connection with estimates of natural selfing.

Methods

Buds were bagged and later repeatedly self-pollinated by anther, as the flowers developed. This was done in the tree bank from 1968 to 1972, some clones being tested more than once during this period (bags 200 and 319, 1968; 500, 1970; 597, 1971, and 600, 1972).

The self-pollinated capsules were reaped, as well as some from open-pollination and the seed was placed in the Jacobsen apparatus and the germinations were counted.

Table 21

Incidence of self-fertility among clones, numbers of flowers pollinated, capsules reaped and selfed seeds per capsule

Clone/ year	Numbers of selfed flowers pol./ capsules reaped	capsules used/ with seed	seeds total	per cap.
G8/72	33/6	6/0	0	
G37/68	74/64	62/9	27	<1
/70	56/27	16/0	0	
G11/70	36/27	20/3	3	<1
G20/70	46/42	20/5	5	<1
G16/70*	20/6	6/1	3	<1
/72	12/4	4/0	0	
G22/70	30/24	20/3	3	<1
G62/72	28/14	14/6	8	<1
G45/70	23/23	16/1	1	<1
G101/70	63/28	16/7	10	<1
G35/68*	73/49	13/4	9	<1
G26/70*	57/11	11/6	9	<1
/72*	37/10	10/7	11	
G50/68	41/34	12/	16	<1
/70	21/9	9/0	0	
G28/70*	43/19	19/13	19	1
G29/70*	45/41	34/17	42	1
G23/70*	50/20	20/8	15	2
/72*	27/18	18/15	41	
G38/70*	55/27	16/15	32	2
G39/70	46/4	3/0	0	2
/71*	42/23	23/15	57	
G68/72	20/16	16/13	31	2

Clone/ year	Numbers of selfed flowers pol./ capsules reaped	capsules used/ with seed	seeds total	per cap.
G7/72	21/1	1/1	3	3
G18/70*	22/14	14/10	36	3
G9/70*	68/54	16/15	48	3
G24/70*	34/34	16/16	52	3
G44/70	36/36	16/4	50	3
G58/70*	47/44	16/15	50	3
G30/70*	41/35	16/12	58	4
G36/71*	16/13	13/	58	4
G14/70*	56/8	8/7	38	5
G63/72	15/3	3/3	14	5
G66/72	29/28	28/26	135	5
G60/70	23/23	16/14	80	5
G21/70	31/2	2/2	12	6
G4/70*	29/29	20/20	157	8
G1/70*	57/8	8/7	68	9
G13/70*	48/32	20/12	176	9
G47/70	61/19	16/15	136	9
G61/72*	24/19	19/19	183	10
G34/70	56/48	16/16	162	10
G64/71*	7/5	5	71	13
/72*	12/12	9/9	115	
G3/70*	35/32	20/17	388	19
G65/72	32/32	10/10	238	24

*Abnormalities were observed among the progeny of these clones, as recorded in Table 10, page 54.

The germinating seeds, or a randomly taken number of them were transferred to soil in tubes (p. 25) and were later planted at close espacement for observation of abnormalities.

Results

The results are recorded in Table 21. Selfed seed was obtained in each of the five main clones during tests of relative yield and in all but one of the other 40 clones listed in the table, i.e. in 44 out of 45 clones tested. In G16, 37, 39 and 50, selfed seed was obtained on one occasion but not on another. Apparently this may be a seasonal effect, or may be associated with the age of the clone, the flowers used, or the time of year when the pollination was done. In G8, which was not pollinated a second time, no seed was obtained after selfing.

The yield of seed per capsule varied greatly between clones, ranging from nought to 24, and although it is not permissible to make close comparisons, since pollinations were not done at comparable stages of receptivity, it is clear that, as in the case of relative yield, a considerable range in self-fertility between clones is indicated.

Some of the clones listed in Table 21 were also used in the later tests of relative yield (lower part of Table 20, page 87) and results of these two are compared for the clones concerned as follows:–

G24, selfed	seeds per capsule	/	relative yield	= 3/7
G9	ditto	/	ditto	3/19
G1	ditto	/	ditto	9/30
G30	ditto	/	ditto	4/32
G34	ditto	/	ditto	10/35
G13	ditto	/	ditto	9/47

The numbers of seeds per capsule after selfing do not correspond well with the separate tests of relative yield, but a closer relationship would be expected if all pollinations in the former tests had been done under comparable conditions. In that case a rough idea of the relative degree of self-fertility among the various clones might be obtained from the simpler procedure of merely self-pollinating. On this basis the majority of the clones listed in Table 21 could be regarded as acceptable in this respect, although as already mentioned in this connection on page 89, consideration would be given to rejection of some of those with higher numbers of selfed seeds per capsule.

The clones tested represent a sample from a wide range of sites in South Africa and the conclusion that most of them are to some extent self-fertile is contrary to reports of some authors in other countries, e.g. Krug & Alves (1949). In this connection it is noted that, as stated by Fryxell (1957), the breeding system of a species may vary with geographical and climatic changes.

The data recorded in Table 21 on numbers of flowers pollinated, capsules reaped and the numbers of capsules with seed, are commented on below.

8.4 Inbreeding effects

The effects referred to here are additional to abnormalities (p. 51) and seed set (p. 83). They are those seen in the first generation from selfing and are concerned with percent fruit set, capsule percent, survival, height depression, bole straightness and combined effects.

The intention is mainly to compare the results of selfing with those from crossing and although open- and mixed-pollinations are also included in some of the tables, these figures are not used much here.

The plants observed are mainly from the tests listed in Table 19 (p. 82) with some reference to those in Table 21, and the methods are therefore as employed under those headings, with the results shown below.

Effect on percent fruit set

The data for this subject, also for capsule percent and survival are recorded in Appendix 4 and Table 22.

Some consideration is given to fruit set even though it is not feasible to be precise about the cause of capsule loss (p. 43). The values after self-pollination were lower than those after cross-pollination in some cases, though not in all. A similar result is represented by the figures in Table 21 where, although no cross-pollinations were done, fruit set after selfing amounted to 100% in G4, 24, 44, 45, 60, 64 and 65, but was very low in some of the other clones.

It is concluded that reduced fruit set was an inbreeding effect in some clones, though not in all.

Effect on capsule percent

This refers to the number of capsules found to have seed, expressed as a percentage of the total number used.

Table 22

Inbreeding effects showing values for fruit set, capsule percent and survival, mainly comparing self-pollination and cross-pollination treatments. (These three variables are grouped together in one table since results lack consistency and are of minor importance)

Test[1]	Year	Cross[2]	% Fruit set		Capsule percent[4]				Chi-square[5] and conclusions	Percent survival in nursery		
			S	X[3]	M	O	S	X[3]		O	S	X[3]
1	'67	6x19	64	60		100	14	86				
5	'68	..	53	38		94	76	67		39	53	35
12	'69	..	66	67		47	22	43		72	80	79
13	'70	..	55	52	0		0	14		83	87	78
			60	56		76	32	45	1,22[NS]	69	74	59
3	'67	10x17	100	93		100	91	100		100	98	89
9	'68	10x15	66	100		87	76	100		94	84	96
17	'70	10x17	91	96	74		30	54		89	73	93
			78	97		91	69	80	0,38[NS]	92	82	93
8	'68	15x17	91	91		48	78	100		87	85	56
10	'68	15x10	68	78		93	67	100				
15	'70	15x17	100	96	83		83	100		88	90	90
			84	89		73	76	100	2,11[NS]	87	87	68
4	'67	17x10	23	92		83	40	100				
7	'68	17x19	26	85		67	25	96		81	100	78
11	'68	17x6	41	67		100	39	100				
16	'70	17x15	86	55	96		72	100		88	82	93
			43	71		94	50	99	11,27**	86	84	90
2	'67	19x6	100	100		58	50	82				
6	'68	19x17	89	88		90	87	100		79	76	54
14	'70	19x6	58	100	100		61	100		59	58	91
			77	94		69	76	97	1,27[NS]	73	73	60
18	'71	58x19	33	61	100	100	74	100	0,46[NS]	92	52	89
20	'72	1x5X	52	53	93		100	100				
21	'72	30x5X	54	83	100	100	100	100				
23	'72	24x5X	30	71	100	100	75	100				
24	'72	13x5X	67	52	100	100	100	100				
25	'72	34x5X	30	46		100	100	100				
26	'72	9x5X	57	35		100	100	100				

[1] The numbered tests refer to those listed in Table 19, page 82.

[2] 5X, cross-pollination using pollen from 5 different clones mixed in approximately equal proportions.

[3] S, self-pollination; X, cross-pollination using pollen from a single clone; M, mixed-pollination using the self and cross pollens mixed in equal proportions by weight and O, open-pollination.

[4] Capsule percent refers to the percent capsules having seed.

[5] Values for Chi-square and conclusions testing the null hypothesis of no difference between the results of self- and cross-pollination in capsule percent.

[NS] Not significant. **Significant at 1 percent level.

In Tests 13 to 17, figures for percent fruit set and capsule percent are from Tests 13A to 17A and for survival they are from Tests 13B to 17B (p. 83).

Considering Test 1 to 18 in Table 22, capsule percent was less in the self than the cross for each individual entry except for Test 5, and without exception for each of the totals by clones. However, using the figures in Appendix 4, the results shown in the table of a Chi-square test (with one degree of freedom and adjusted for continuity) indicate no significant difference at the 5% level between the self and the cross totals for individual clones, except in the case of G17. In some cases this was no doubt due to lack of power because of the small sample size.

Again, however, there was no difference between the self-pollinated and the cross-pollinated progeny in most of the clones in tests numbered 20 to 26, where there were figures of 100% after both these treatments in five tests out of six.

In addition under the heading "capsules used/with seed" in Table 21, some of the clones had figures after selfing amounting to 100% and it is concluded that there was an inbreeding effect on capsule percent in some clones, but not in others.

Effect on survival

It might be expected that survival in self-fertilised plants would be poorer than in those derived from cross-fertilisation, but from the figures in Table 22, this was not generally the case in the nursery, i.e. for the first three to five months after sowing, the reverse being quite commonly true.

It was observed repeatedly during the course of these tests that in the crosses (with large numbers of seeds per capsule and protracted germination time, page 113), the later germinations were very likely to die shortly after sowing the seed and this is put forward as one likely explanation for the lower survival in the crosses, where this is shown in the table.

In any case, the results are inconsistent and for this reason survival figures are omitted from Tests 20 to 26 in Table 22. It is unprofitable to pursue this matter further, except to mention that by some eight months after planting, the survival was poor in selfs exhibiting certain of the deviant types listed in Table 10 (p. 54).

Effect on mean heights

Measurements of trees planted in randomised blocks were made by the method described on page 85 and resulted in the mean heights from self-, open- and cross-pollination shown in Table 23. Mean heights from mixed-pollinations are shown in Table 32, page 118.

Table 23

Inbreeding effects showing mean heights in metres after self-, cross- and open-pollination treatments, range in heights and percent height depression

Test[1]/ year Cross	Reps[3] & age in months	Treatments[4] & nrs. measured	Mean height m	t[5] and conclusions	Range in individual heights	%height[6] depression
3/67	4x5	S 19	,93	7,61**	,81	S/X −49
10x17	17	X 19	1,83		1,56	S/O −54
		O 20	2,02		3,11	O/X +10
5/68	4x10	S 36	2,02	7,25**	2,58	S/X −32
6x19	18	X 39	2,96		2,5	S/O −8
		O 36	2,19		2,95	O/X −26
6/68	5x10	S 50	3,84	3,88**	2,85	S/X −13
19x17	18	X 49	4,4		3,3	S/O −10
		O 50	4,27		5,78	O/X −3
7/68	nil	S 3	2,07			
17x19	18	X 4	2,9			
		O 3	2,48			
8/68	5x10	S 42	2,91	8,58**	4,7	S/X −44
15x17	17	X 46	5,21		3,0	S/O −31
		O 47	4,22		6,09	O/X −19
15B/70	4x10	S 38	1,04	5,82**	1,76	S/X −38
15x17	14	X 40	1,67		1,2	
18/71	5x10	S 46	1,6	3,35**	1,8	S/X −18
58x19	15	X 46	1,95		2,15	S/O −16
		O 48	1,91		2,25	O/X −2
19/71[2]	11x10	S 108	1,45	8,88**	3,2	S/X −31
1x5X	14	X 108	2,09		2,0	S/O −15
		O 107	1,7		3,1	O/X −19
20/72	6x10	S 60	1,87	7,32**	2,35	S/X −26
1x5X	11	X 60	2,53		1,3	S/O −20
		O 59	2,34		2,35	O/X −8
21/72	7x10	S 65	1,26	10,15**	2,35	S/X −46
30x5X	11	X 68	2,34		2,3	S/O −34
		O 69	1,9		2,6	O/X −19
24/72	9x10	S 88	1,42	10,83**	2,35	S/X −41
13x5X	13	X 90	2,39		2,0	S/O −34
		O 87	2,14		3,25	O/X −11
25/72	6x10	S 55	2,22	2,02*	2,4	S/X −8
34x5X	13	X 56	2,4		1,7	S/O −3
		O 52	2,29		1,75	O/X −5
26/72	3x10	S 30	1,88	6,08**	1,75	S/X −27
9x5X	13	X 30	2,59		1,6	S/O −13
		O 30	2,17		3,2	O/X −16

[1]The numbered tests refer to those listed in Table 19, page 82.

[2]5X, cross pollination using pollen from five different clones mixed in approximately equal proportions.

[3]In each test, the upper line shows the number of replications (of plots in randomised blocks) times the number of trees in each; the lower line shows the age when heights were measured, in months from the time the seed was sown.

[4]S, self-pollination; X, cross-pollination and O, open-pollination.

[5]Values for Student's t and conclusions comparing mean heights of self- and cross-pollinated progeny.

*Significant at 5 percent level. **Significant at 1 percent level.

[6]Depression in mean height of progeny from pollination treatments, S/X = the difference between the cross and the self over the cross times 100. Similarly S/O is the self compared with the open and O/X is the open compared with the cross.

At age 11 to 18 months after the seed was sown, mean heights after self-pollination were less than after cross-pollination in each of 12 replicated tests, also in the unreplicated test number 7. Comparing the results of these two treatments by a t-test for two independent samples (Snedecor & Cochran 1969), differences were below the 1% level of significance in eleven tests out of twelve and at about the 5% level in the remaining test. In the former, differences as large as those observed would only occur by chance once in over 100 times and it is concluded that height depression was a consistent inbreeding effect.

In tests where appreciable numbers of clearly recognisable deviant types developed, these were largely responsible for the reduced mean heights, e.g.:—

Test 8	: G15: deviant type 10:		9	deviant self	,25	m
			33	normal . .	3,63	
			46	normal cross	5,21	
Test 19	: G1	3	18	deviant self	,88	
			90	normal . .	1,56	
			108	normal cross	2,09	
Test 21	: G30	5&11	27	deviant self	,58	
			38	normal . .	1,74	
			68	normal cross	2,34	

With the above height depressions and poor vigour of deviants, one would expect the range in individual heights after crossing to be less than that in the other two treatments. This is confirmed in the column headed "Range" in Table 23, where the figure is usually least in the cross-pollinated treatments.

The measurements were used to calculate percent height depression, e.g. the item S/X in the table represents the depression in mean height after self-pollination compared with cross-pollination expressed as a percentage of the latter. The height depression S/X varied from 8% in G34 to 49% in G10. The depressions S/O were usually less pronounced.

Comparing the results of open-pollination with those from crossing, the mean heights in the table indicate, for the stated age, the order of depression to be expected in the natural progeny, compared with what might be obtained if hand-pollination were to be practised, using the pollen of the tests. Apart from Test 3, where the open-pollinated mean height was the highest of the three treatments, depressions in the opens compared with the various crosses varied from 2% in G58 to 26% in G6.

The values for open-pollination in relation to the self and cross are considered on page 107 in connection with the use of height in estimating percent selfing.

From the above and other results, G19 emerges as a desirable clone because of its low relative seed yield after selfing compared with crossing, little in the way of deviant types of a detrimental nature among the progeny and a generally lesser inbreeding depression in height.

In addition to the above height measurements, for the purpose of the investigations on page 99, a rough assessment of relative dominance was made as described on page 86. The stands were at age about 3 years, and inspection of the figures in the footnotes to Table 24 (p. 98), reveals that the height depressions shown above were still present.

There were also corresponding diameter differences as illustrated in Figure 38 (facing p. 53), where the lines of trees from self-pollination and from cross-pollination in Block 2 of Test 17 are shown at age 3 years. The mean breast height diameters of these were self 6,4 cm and cross 11,5 cm, and the two trees nearest to the camera measured 6,0 cm and 11,5 cm respectively.

Effect on bole straightness

Some trees derived from selfing are markedly inferior in straightness compared with the crosses, but on the other hand certain selfed individuals are among the straightest to be found and it is not clear whether inferior stem form is to be regarded as one of the inbreeding effects. It became possible to investigate this as soon as certain of the tests recorded in Table 23 had been established long enough for it to be possible to prune the dead branches, thus permitting a clear view of the boles. Observations were then made at age three years from sowing (p. 86).

The self- and cross-pollination treatments being compared represent independent samples, the level of measurement is ordinal at best and the Mann-Whitney U test for large samples (n_2 more than 20) (Siegel, 1956) was used to test results, with significance level set at 0,05 for a one-tailed test. The null hypothesis H_o is that the self- and cross-pollinated samples are equal in regard to straightness and H_1 is that the crosses are better than the selfs.

To compare the self- and cross-pollination treatments, the mean scores (rating for straightness) for these two are shown as percentages in Table 24. From the score for each individual tree, ranks were assigned starting with rank one for the lowest

Table 24

Inbreeding effects showing scores for bole straightness and for straightness combined with relative dominance after self-, cross- and open-pollination treatments at age three years

Test/ year and cross	Nr.[1] trees	Straightness[3] score[2]	R_1	U	z and conclusions	Combined[4] score[2]	R_1	U	z and conclusions
5/68 6x19	S 36	54,2%	1441	629	,77[NS]	51,8%	1175,5	894,5	2,04*
	X 39	51,5				67,8			
	O 36	50,6				56,6			
6/68 19x17	S 50	33,4%	3372,5	302,5	6,5**	58,2%	2919	756	3,2**
	X 49	58,2				75,6			
	O 50	46,2				67,6			
8/68 15x17	S 42	40,0%	1643,5	1191,5	1,88*	50,7%	2774,5	468,5	4,7**
	X 46	52,2				75,2			
	O 47	44,5				62,0			

[1]Number of trees per treatment after self- (S), cross- (X) and open-pollination (O).

[2]The mean score for each treatment expressed as a percentage.

[3]Values for R_1, U, z and conclusions (Siegel, 1956) are shown for stem straightness, comparing results from self-pollination with those from crossing.

[4]Values for R_1, U, z and conclusions are shown for stem straightness combined with relative dominance (p. 86), comparing results from open-pollination with those from crossing.

[NS]Not significant. *Significant at 5 percent level. **Significant at 1 percent level.

The scores for relative dominance were:–

Test 5/68		Test 6/68		Test 8/68	
S	49,4	S	83,0	S	61,4
X	84,1	X	93,1	X	98,3
O	62,5	O	89,0	O	79,6

score in the two treatments combined. The rankings so assigned to the self and to the cross were then totalled separately and the sum of these for the treatment with the lower number of trees n_1 gave (rank) R_1 shown in the table. The values for n_1 and n_2 are shown under the heading "number of trees". U, the statistic used in this test, was calculated from R_1, n_1 and n_2 and from this the values shown for z were obtained.

In Tests 5,6 and 8 the z values of 0,77 6,5 and 1,88 have one-tailed probabilities under H_o of P=0,22, less than 0,01 and 0,03 respectively. Therefore in the case of Test 5, H_o is accepted, (even when corrections have been made for ties, when the value for z becomes 1,4), but it is rejected in favour of H_1 in the other two tests and it is concluded that inbreeding affected straightness adversely in two out of three tests. In these two tests also, the scores shown for the progeny from open-pollination were intermediate between the self and the cross.

Test 5 contained some exceptionally straight trees among the selfs and the mean score was slightly higher than was the case for the cross. Test 8 included individuals of the deviant type number 10, stem leaning, which received very low scores for straightness.

Combined effects

It is assumed from previous results that there would be significant differences between the self-pollinated and the cross-pollinated progeny and the intention here was to compare the results of open-pollination with those from crossing and to note, as far as possible, the overall inbreeding effect.

For this purpose, the above mentioned straightness was combined with a rough assessment of height, referred to in the footnotes to Table 24 as relative dominance (p. 86), which was assessed at the same time as the straightness. Thus these two features were taken into account, and indirectly, the effect of the presence of any deviant seedlings and their survival prospects. Effects such as those on seed yield and fruit set were not represented.

The points made in connection with straightness also apply here, the Mann-Whitney U test was again used and in this case H_o was rejected in each of the three tests.

The mean scores for the opens were depressed in relation to the crosses by roughly 11%, 8% and 13% in Tests 5, 6 and 8 respectively. This is taken to represent

the amount of degrade in vigour and straightness of open-pollinated progeny resulting from the amount of selfing which occurred.

8.5 Natural selfing

If the species in use has been shown to produce appreciable amounts of viable seed after manipulated selfing, there is cause for concern that the same may also occur naturally in a seed orchard. Depending on the frequency of such selfing, the occurrence of inbreeding depression, its nature and the stage when it is expressed phenotypically, the value of the orchard as a source of improved seed may be affected adversely. The risks involved have been discussed by many workers and it has been pointed out that these risks are all the greater since there are several ramets of each clone in the orchard.

The main means of assessing percent selfing was by the use of genetic markers, but some account was also taken of such variables as mean heights.

Estimates of selfing based on normal to deviant ratios

Methods. Taking into account the results of preliminary tests (Table 10, p. 54, and Table 21, p. 90), in which seedlings derived from selfing (and open-pollination) of 44 clones were examined for abnormalities, those with suitable deviant types among the progeny were chosen for use in more comprehensive tests. These are included among the numbered tests listed in Table 19 (p. 82) where some aspects of the methods employed are described. Although most use was made of the latter, results from the preliminary tests were also usable where, as in the case of clone G61, this was justified by the number of seedlings available for examination.

The estimates of natural selfing were made in proportion according to the ratios of normal to deviant seedlings after open-pollination compared with that after self-pollination. In those tests where, as indicated, more than one deviant type developed (p. 56), all types other than the one under consideration were treated as normal.

Since manipulated self-pollination was done on all the clones in the tree bank, it was possible, from observation of the resulting progeny, to make an assessment of the numbers of carriers likely to be present there.

In Test 8, (Table 25, p. 102), only the seedlings taken randomly and planted in randomised blocks (p. 85) were retained, and were available for further observa-

tion beyond that stage. In other tests used for the present purpose, all surviving seedlings, including those not taken for planting in the randomised blocks were planted during the same season outside the formal lay-out, either as surrounds to the randomised blocks, in unreplicated plots at the usual espacement, or in unreplicated plots at a close espacement of about 0,3 by 1 metres. The latter was done where necessary to find space for all seedlings, which were retained till no further changes occurred in the formation of deviant types, after which the plots were liquidated. All seedlings were taken into account in estimating selfing, on the assumption that the results were not affected by the differences in treatment.

Results. The results are shown in Table 25, where figures for mixed-pollination are included for use in connection with selective fertilisation.

Nothing is known of embryo mortality, nor of the effects which subsequent inviability may have on results. Most of the deviations from normal were not expressed phenotypically till near or after planting time and the estimates are not of the actual number of self-fertilisations, but of the number of self-fertilised plants which survived long enough to be classified.

It is also noted that the usefulness of the markers is subject to limitations such as those referred to on page 13, and to the points made in this connection by Fryxell (1957), Sorensen (1967) and Franklin (1971b), (p. 12).

Although some of the deviants formed graded series (p. 51), suggestive of multiple gene inheritance, the ratios in Table 25 are tested for control by a single recessive gene.

Results are considered below according to deviant types (Table 10, p. 54) and test numbers (Table 19, p. 82). The clones which were identified as carriers in the tree bank are recorded in Table 10 and this matter is also referred to below. Where deviants are being considered which occur in the progeny of more than one clone, the stated estimates are regarded as maxima. This is because the open-pollinated progeny would be likely to include some crosses between carriers which would be indistinguishable from selfs.

Type 10. This type usually developed two to eight months after planting, during which period some trees were liable to die from unknown causes.

The defect was not noted in typical form among the selfed progeny of the other clones which were observed long enough for it to appear. Although the clone was probably the only carrier in the tree bank, there were three ramets of the same clone, two of which were adjacent to each other and were used in these tests. Although an over estimate of percent selfing would be expected to result, from a practical point of view, the figures obtained may be representative of conditions in the seed orchard, except that normally none of the several ramets of a clone would be adjacent as in this case.

Table 25

Estimates of percent selfing on the basis of normal to deviant ratios in self-pollinated and open-pollinated progeny (no deviants in the cross, and results of mixed-pollination included here for convenience)

Test[1] & year	Deviant[2] type	Cross[3]	Normal numbers	: Deviant ratio	Chi-square[4] and conclusions	Percent[5] selfing
8	10	15x15	33:9	3,7:1	,13 NS	*
1968	stem	15x0	46:1			10%
	leaning	15x17	46:0			
..	6	15x15	38:4	9,5:1	4,6 *	*
	leaves	15x0	46:1			22%
	small	15x17	46:0			
15B	10	15x15	84:18	4,7:1	2,6 NS	*
1970	stem	15x0	153:8			28%
	leaning	15x17	91:0			
		15xM	171:3			
22[6]	..	15x15	26:1			
1972		15x0	114:11			
		15x5X				
		15xM	134:3			
19	3	1x1	637:165	3,9:1	8,1**	
1971	leaf	1x0	522:108			83%
	margins	1x5X	512:0			
	irregular					
20	..	1x1	56:18	3,1:1	NS	
1972		1x0	119:12			38%
		1x5X	69:0			
		1xM	152:7			
27	..	1x1	152:44	3,5:1	,56 NS	
1972		1x0	183:12			27%
21	5	30x30	49:25	2:1	2,6 NS	*
1972	leaves	30x0	86:8			25%
	curved	30x5X	155:0			
		30xM	191:6			
..	11	30x30	64:10	6,4:1	4,6*	*
	procum-	30x0	90:4			32%
	bent	30x5X	155:0			
		30xM	196:1			
24	5	13x13	161:44	3,7:1	1,2 NS	*
1972	leaves	13x0	147:10			30%
	curved	13x5X	90:0			
		13xM	185:6			
..	11	13x13	191:14	13,6:1	35**	
	procum-	13x0	154:3			*
	bent	13x5X	90:0			28%
		13xM	190:1			
..	7	13x13	185:20	9,3:1	25**	
	leaves	13x0	152:5			33%
	dark	13x5X	90:0			
		13xM	190:1			
Prelim.[7]	4	61x61	123:32	3,8:1	1,3 NS	
test	leaves	61x0	156:6			18%*
1972	crowded					

[1] See numbered tests, Table 19, page 82 .

[2] See deviant types, Table 10, page 54 .

[3] 5X, cross-pollination using pollen from five different clones mixed in approximately equal proportions; M, mixed-pollination using the self and cross pollens mixed in equal proportions by weight and O, open-pollination.

[4] Chi square and conclusions, testing the null hypothesis of no difference between the normal to deviant ratios observed after self-pollination and a ratio of three normal to one deviant.

NS Not significant. *Significant at 5 percent level. **Significant at 1 percent level.

[5] Figures for percent selfing which are marked with an asterisk are likely to be over-estimates because of the presence of other carriers detected in the tree bank.

[6] Test 22, no entry made for percent selfing since results are unreliable because of the small numbers of selfed progeny.

[7] Prelim. test, this refers to the preliminary test of G61 (Table 21, p. 90).

Of the three tests shown in the table and done in three separate seasons using G15, Tests 8 of 1968 and Test 15 of 1970 gave figures for selfing after open-pollination of 10% and 28% respectively, with corresponding normal to deviant ratios in the selfs of 3,7:1 and 4,7:1. The 28% in 1970 is likely to be the more reliable because of the greater number of plants observed. In Test 22, not only was the deviant type less clearly defined than had previously been the case, but also the self-pollination resulted in only 40 germinations and of these only 27 survived long enough to be classified. If the ratio of 26:1 obtained for this small number were to be used, a figure of over 100% would result and hence no entry is made in the table for percent selfing in this case. If the ratio of 84:18 of Test 15 is applied here instead, a figure of 50% is obtained, although not much reliance can be placed on this.

As recorded in Table 10, page 54, two phenotypically different types occurred in the progeny of G15 in addition to number 10. One of these was Type 8 with dense foliage which, in each of the three tests with this clone, only occurred after open-pollination. There was also Type 6 with small leaves which was only clearly different from normal in Test 8.

In calculating percent selfing on the basis of the ratios of Type 10, the above two types were treated as normal (p. 100) as was done when using Type 6 in Test 8, the ratios of which gave 22% selfing.

In Tests 8 and 15, there was no evidence against a hypothetical ratio of 3:1 in Type 10 and the parent could be heterozygous for a single recessive gene (p. 56). The ratio was higher than 3:1 in each case, possibly due to higher embryo mortality among the deviants (Sorensen, 1967).

Type 3. This appeared in G1 from the seedling stage onwards and could have been classified fairly well in Test 19 on the basis of the first pair of seedling leaves only, although three extra deviants among the self-pollinated progeny and two after open-pollination were seen by planting time at age four months and by this time also, three selfs and one open had reverted to normal and some of the deviants had died.

No other family was seen with this type of deformity and since there was only one ramet of the clone in the tree bank, all the deviants can be taken to represent selfs and the results express what could be expected without any other ramets of the same clone in a seed orchard.

As a result of observations of a preliminary test of G1 becoming available late in the 1971 season, the clone was examined and found to have a few flowers left which were used for Test 19 in April/May, well outside the main flowering season and at a time when there were very few other flowers in the tree bank. It was recorded that floral development was noticeably retarded at this time of the year compared with what had been found to be normal (p. 30) and as stigmas were very slow in becoming receptive, self-pollination had to be done over a much longer period than usual.

Tests 20 and 27 were done during the main part of the flowering season, Test 20 at the beginning and Test 27 more towards the end of the season.

A high figure for percent selfing would naturally be expected from flowers which opened out of season and the 83% obtained in Test 19 confirmed this.

The relatively low figures of 38% and 27% for Tests 20 and 27 give, by contrast, an estimate of the amount of selfing for the main part of the season.

The normal to deviant ratios in the self-pollinated progeny of the three tests ranged from 3,1:1 to 3,9:1 and were reasonably consistent. There was no evidence against a hypothetical ratio of 3:1 in Tests 20 and 27, but there was in Test 19. This anomalous result is no doubt connected with the much larger number of plants used in the latter test.

Type 5. In this type, the leaves became curved downwards at or near planting time. It was not always easily classified and sometimes became similar in appearance to Type 4. In addition to difficulty experienced in classifying seedlings in some cases, the results are likely to be on the high side because of the considerable number of other carriers recorded in Table 10, page 54.

Of two tests which were done in 1972 and which showed deviant plants of Type 5, numbers 21 of G30 and 24 of G13, the latter had been expected from the preliminary trials shown in Table 21 to yield seedlings of Type 4. In addition, Types 7 and 11 appeared in these tests and are included below under the present heading for Type 5. The indistinct Type 15 with pale green foliage after planting was also seen.

In Test 21, three months after planting, out of 25 trees from selfing which had been classed as Type 5, one remained in that category, three died, 11 reverted to normal, two became dwarf plants and four became similar to Type 4, while the remaining four (as well as others previously classed as normal) changed to Type 11 (procumbent).

Similarly in Test 24, out of 44 of Type 5, ten remained unchanged, one died, 14 reverted to normal, 12 became procumbent and seven (as well as others previously classed as normal) developed into Type 7, with dark broad leaves.

In Test 21 of G30, selfing was 25% using Type 5 and 32% using Type 11.

In Test 24 of G13, the two corresponding figures were 30% and 28%. Using Type 7, which was relatively well defined in this case, the estimate was 33%.

There was no evidence against a hypothetical ratio of 3:1 for Type 5, but there was in the case of Types 11 and 7.

Type 4. The remarks in this case are similar to those for Type 5, although the effects on vigour and survival were sometimes more detrimental. Test 23 of G24 had been expected, from preliminary trials, to exhibit this type, but no deviant seedlings appeared and the test is not used here. However, considerable numbers derived from a preliminary test of G61 are made use of. In this test, the deviants were clearly defined Type 4, except for three which developed an appearance rather more like that of Type 5. Counting all as Type 4, the ratios obtained and shown in Table 25 at age seven months after sowing were 123:32 after self-pollination and 156:6 after open-pollination, giving 18% selfing.

There was no evidence against a hypothetical ratio of 3:1 in this test.

Type 9. This developed in G17 several months after planting. The ratios obtained are shown in Table 10 (p. 54), and these would result in an estimate of about 100% natural selfing. This seems very unlikely to be correct, especially in view of the repeatedly low relative yields for this clone shown in Table 20 (p. 87), but in any case, the type was often too indefinite to give reliable results.

Estimates of selfing on other bases

The bases referred to are those evaluated in Tables 22 (p. 93) and 23 (p. 95), where the results of self-, open- and cross-pollinations are listed. The main object here was to attempt to draw some conclusions from the values after open-pollination in relation to the other two treatments.

The figures for fruit set are not considered because of the uncertainty about the cause of capsule losses (p. 43), while those for survival in Table 22 were inconsistent. As regards capsule percent, the figures after open-pollination were intermediate between those for the self and the cross in six tests out of 18, indicating in these cases that the capsules derived from open-pollination consisted of a mixture from selfing and from crossing.

Table 26

Estimates of percent selfing comparing results from normal to deviant ratios in self-pollinated and open-pollinated progeny, with results from mean heights of these progeny

Test[1]	Deviant[2] type	Cross[3]	Reps.[4]	Ratio normal: deviant	% Selfing	Mean heights in formula X–O/X–S[5]	% selfing
8	10	15x15	5	33:9		5,21–4,22/5,21–2,91	
		15x0		46:1	10%		43%
		15x17		46:0			
15B	10	15x15	4	28:10		1,67–1,19/1,67–1,04	
		15x0		33:4	41%		76%
		15x17		40:0			
19	3	1x1	6	42:17		2,12–1,79/2,12–1,35	
		1x0		51:6	37%		43%
		1x5X		58:0			
20	3	1x1	6	46:14		2,53–2,34/2,53–1,87	
		1x0		55:5	36%		29%
		1x5X		60:0			
21	5	30x30	7	44:21		2,34–1,90/2,34–1,26	
		30x0		60:8	36%		41%
		30x5X		68:0			
24	5	13x13	9	65:25		2,39–2,14/2,39–1,42	
		13x0		80:9	36%		26%
		13x5X		90:0			

[1]See numbered tests, Table 19, page 82 .

[2]See deviant types, Table 10, page 54 .

[3]5X, cross-pollination using pollen from five different clones mixed in approximately equal proportions and O, open-pollination.

[4]Number of replications of ten-tree, line plots.

[5]Pollination treatments, cross minus open, over cross minus self.

The same applied to mean heights, although the figures were much more consistent, since in eleven replicated tests out of twelve, selfs were least and the opens intermediate. It is therefore of interest to see how the result of applying mean heights in the expression X–O/X–S (pollination treatments cross minus open, over cross minus self, Franklin 1971a) compares with that derived from deviant progeny.

For this purpose, the normal to deviant ratios used exclude those planted outside the randomised blocks (p. 85), since the calculation of mean heights was confined to trees in those blocks (p. 94). Also, in the case of Test 19, the open pollinated seedlings which were taken randomly for planting in the randomised blocks included such a high proportion of deviants that selfing would have worked out at over 100% and, for the purpose of this exercise, the proportion of deviants was reduced to a more likely figure by disregarding blocks 2,3,5,6 and 10.

As shown in Table 26, in two tests of G15, the formula gave percentages which differed widely from those based on normal to deviant ratios, but in the other four tests, in which mixed cross pollens were applied, the two sets of figures are in agreement to within 10 percent or less. Assuming that the ratios gave reliable results, the indication from these few tests is that a comparison of mean heights in the three treatments could give an indication of the amount of selfing, though this might not always be reliable. Probably much would depend on the extent to which the pollen applied in cross-pollination corresponds in its effect, with that which was involved in nature.

Another possibility would be to compare results of natural selfing with estimates of the relative self-fertility of the various clones, based either on the relative yields after selfing compared with crossing (p. 83), or from selfed seed yields alone (p. 89).

It was shown on page 91 that these two did not correspond well, although it was suggested that the latter might give a more reliable figure if all self-pollinations were done under comparable conditions. The more time-consuming calculation of relative yield (all germinations) would be expected to be more representative and below, these figures are placed alongside those for percent selfing (surviving seedlings only), to see whether they correspond in regard to order:–

G13 Test 24	relative yield/percent selfing	47/30 (Type 5)
G30 . . 21	. .	32/25 . .
G 1 . . 20	. .	30/38 (Type 3)
G15 . . 15	. .	19/28 (Type 10)
G15 . . 8	. .	8/10 . .

The result is again not very encouraging, possibly because the samples are not identical. Presumably it can at least be said for the relative yield figures that provided clones are tested under comparable conditions (and using the same pollen throughout), an indication of relative self-fertility would be obtained which could be used in considering rejection of the more self-fertile clones, especially those also subject to much inbreeding depression.

In this connection it is assumed that where seed yields are consistently low after manipulated selfing, the same would usually apply in nature, but where high, the issue would be in doubt because the result of separate self- and cross-pollinations would not take into account possible selective fertilisation and other controlling factors which only have effect in nature.

8.6 Barriers to selfing

As continuation to the remarks on page 79, some further investigations were made into the subject of barriers by comparing the number of germinations of pollen on the stigma after self-pollination compared with those after cross-pollination, and by investigating selective fertilisation.

Incompatibility on the stigma

The numbers of pollen germinations comparing the results from selfing with those from crossing were counted 10 to 15 hours after pollination, the intention being to investigate possible incompatibility on the stigma.

Methods. The procedures for preparation of the stigmas for examination were the same as those already described on page 72.

Three separate pairs of reciprocal crosses were done by brush at the tree bank in 1970, using receptive stigmas, the flower buds having been emasculated and bagged in advance. Pollen tests were done in the same way as for relative yields (p. 84), although no Chi-square tests were applied in the present case.

Results. Details of results are shown in Appendix 5 and these are summarised in Table 27, under tests numbered 1 to 3, where the factors shown in the table are derived from the results of the pollen tests shown in the appendix.

Although the time from pollination to immersion in xylol should be the same for each stigma, this was not achieved very accurately in Test 1 and it is not known

Table 27

Incompatibility on the stigma from counts of germination of pollen grains on receptive stigmas after self-pollination compared with cross-pollination

Test	Cross	Germinations per stigma			Cross	Germinations per stigma		
		u[1]	factor[3]	a[2]		u[1]	factor[3]	a[2]
1	15x15	3,2		3,2	19x19	4,4	x1,7	7,5
	15x19	1,3	x1,7	2,2	19x15	17		17
2	10x10	119,2	x1,04	124	19x19	8,5		8,5
	10x19	51,4		51,4	19x10	56,2	x1,04	58,4
3	10x10	192,2		192,2	15x15	156	x11,2	1 747,2
	10x15	87,3	x11,2	977,8	15x10	243,7		243,7

[1]unadjusted.

[2]adjusted according to results of pollen tests in Appendix 5.

[3]the factors shown are derived from results of the pollen tests.

to what extent differences of about half an hour, which occurred in that test, may have affected the results.

In the three reciprocal tests, considering the numbers of germinations per stigma after self-pollination compared with cross-pollination, there was no consistent difference. The two treatments were each better in three cases and this applied whether or not the results of the pollen tests were taken into consideration and if any degree of incompatibility was present at this stage, it was not consistent. In any case, appreciable numbers of germinations occurred in each of the self-pollinated treatments.

Although other possibilities such as inhibition of growth of pollen tubes in the style were not examined, the reduced seed yield after selfing compared with crossing is referred to tentatively as being due to some self infertility (as is common in other tree species), rather than to incompatibility. The possibility of the latter nevertheless remains and in fact, the low selfed seed yield at early flower stages (p. 66) is rather suggestive of it.

Selective fertilisation

This subject was considered in the broad sense referred to by Squillace & Bingham (1958) and the tests done and treatments applied were the same as for natural selfing, with the addition of mixed- (self and cross)-pollination in equal proportions. This equality was arranged only approximately in Tests 9 to 12, but this was later considered inappropriate and thereafter, the pollens were mixed in equal proportions by weight (p. 83).

Four possible means of assessment were considered namely early vigour (sub headings i and ii), deviant progeny, seed yields per capsule and mean heights, as dealt with below.

Tests based on early vigour are described under two sub-headings (i) and (ii) because of the need, discovered later, for arranging comparable numbers of seeds per capsule in each of the treatments.

Selective fertilisation based on early vigour (i)

Sub-heading (i) refers to tests in which no attempt was made to arrange equality in numbers of seeds per capsule in the various treatments. Tests 3 to 12 (Table 19, p. 82), done mostly without mixed-pollination treatments, served as a starting point.

Table 28

Assessment of selective fertilisation based on mean seed germination time and mean epicotyl length after self-pollination compared with cross-pollination

Test[1]/ year cross	Mean germination time (Nr.) days	Mean epicotyl length (Nr.) cm	age[2]	Test[1]/ year cross	Mean germination time (Nr.) days	Mean epicotyl length (Nr.) cm	age[2]
5/68				8/68			
6x6	(138) 4,4	(73) 1,3	4	15x15	(121) 5,5	(95) 2,5	3
6x19	(198) 6,3	(69) 2,1		15x17	(926) 7,1	(107) 2,0	
12/69				7/68			
6x6	(55) 9,4	(44) 4,9	3	17x17	(5) 4,4	(4) 1,5	3
6x19	(138) 12,2	(109) 4,2		17x19	(572) 4,8	(18) 1,4	
3/67				11/68			
10x10	(99) 7,9	(49) 1,7	5	17x17	(18) 3,9		
10x17	(630) 4,7	(55) 1,3		17x6	(46) 7,2		
9/68				6/68			
10x10	(57) 4,0	(46) 2,2	3	19x19	(87) 5,1	(63) 1,6	3
10x15	(1149) 9,1	(79) 1,2		19x17	(1370) 10,2	(100) 1,2	

[1] See numbered tests Table 19, page 82 .

[2] The column headed age shows the age when epicotyl length was measured, in months from the time when the seed was placed for germination.

In Test 9, the recording of one germination date in the cross was omitted in error.

In Test 11, 46 germinations are shown (not 752 as in Appendix 4), since seeds from some capsules were randomly excluded from the calculation of germination time (p. 84).

Table 29

Variation in mean seed germination time with changes in numbers of seeds per capsule

Test[1]/ year cross[2]		Numbers of seeds/capsules = seeds per cap.	Mean germin. time days
13B/70			
6x6	All[3]	194/20 = 9,7	8,9
	Sel.	77/12 = 6,4	8,2
6xM	All	59/10 = 5,9	6,6
6x19	All	97/11 = 8,8	9,5
	Sel.	54/9 = 6,0	8,4
6x0	All	269/16 =16,8	10,6
	Sel.	44/7 = 6,3	7,5
15B/70			
15x15	All	119/22 = 5,4	6,0
15xM	All	193/24 = 8,0	5,2
	Sel.	98/19 = 5,2	4,7
15x17	All	101/12 = 8,4	4,9
	Sel.	58/10 = 5,8	4,8
15x0	All	193/13 =14,9	6,3
	Sel.	54/6 = 9,0	5,9
17B/70			
10x10	All	97/30 = 3,2	3,7
	Sel.	55/12 = 4,6	3,7
10xM	All	99/22 = 4,5	3,5
	Sel.	52/13 = 4,0	3,2
10x17	All	57/8 = 7,1	3,7
	Sel.	41/7 = 5,9	3,5
10x0	All	118/28 = 4,2	3,6
	Sel.	57/13 = 4,4	3,5
14B/70			
19x19	All	19/14 = 1,4	6,6
19xM	All	69/12 = 5,8	8,6
	Sel.	20/8 = 2,5	6,7
19x6	All	35/4 = 8,8	7,3
19x0	All	34/8 = 4,3	7,3
	Sel.	21/7 = 3,0	6,6
16B/70			
17x17	All	57/19 = 3,0	4,8
	Sel.	41/9 = 4,6	4,8
17xM	All	65/9 = 7,2	4,1
	Sel.	43/8 = 5,4	4,1
17x15	All	91/11 = 8,3	4,1
	Sel.	42/8 = 5,3	4,2
17x0	All	135/16 = 8,4	4,5
	Sel.	48/10 = 4,8	4,1

[1]See numbered tests Table 19, page 82.

[2]M, mixed-pollination using the self and cross pollens mixed in equal proportions by weight and O, open-pollination.

[3]All and Sel. refer to all capsules and selected capsules respectively.

Methods. Early vigour as represented by (seed) germination time and epicotyl length of seedlings three to five months after sowing, was measured by the methods described on pages 84 and 85 respectively.

Using these figures, the values after self-pollination were compared with those after cross-pollination. This was done with a view to making observations on the effects of these two treatments in relation to those on mixed- (self and cross)-pollinated progeny, as done by Barnes *et al.* (1962) with *Pinus monticola.*

Results. The results in Tests 3,5 to 9, 11 and 12 are shown in Table 28. It is seen that mean germination time was less after selfing than after crossing in seven cases out of eight and the selfs were better in epicotyl length in six cases out of seven. This was unexpected and from examination of the figures, it appeared that germination time was longer when there were large numbers of seeds per capsule (as after cross-pollination), compared with capsules having few seeds, as in selfing. There was confirmation of this in that there were strong positive correlations between numbers of seeds per capsule and germination time:—

Test	5	G6x6	r	=	+0,69	P = 1%
	12	G6x19	r	=	+0,97	P = < 1%

Selective fertilisation based on early vigour (ii)

Sub-heading (ii) refers to tests in which, in view of the above results, attempts were made to arrange comparable numbers of seeds per capsule in the various treatments so as to eliminate the effect of this variable on early vigour.

Methods. In addition to the methods referred to in (i) above, in Tests 13B to 17B, pollination was done at "chosen stages" (p. 83), i.e. aiming at the desired result by making use of the changes in receptivity with flower age. But this procedure for equalisation was only moderately successful and it was therefore decided to aim at still closer parity by appropriate selection of capsules.

Results. The results are shown in Table 29, where there are two lines for each test, one showing all capsules and the other showing selected capsules.

In 11 cases out of 16 in Table 29, where the selection of capsules resulted in only slight changes in the numbers of seeds per capsule, there were corresponding changes in germination time. In most cases, germination time appeared to be so sensitive to changes in numbers of seeds that its use for the present purpose is not thought worth considering further. As regards epicotyl length, as seen in Table 28, this feature shows a similar trend to that of germination time in most cases and it was decided that no use would be made of either of these variables.

Table 30

Assessment of selective fertilisation based on the normal to deviant ratios observed in the progeny from mixed-pollination, compared with those expected from separate self- and cross-pollination

Test[1]	Cross[2]	Deviant[3] type	Ratios		Chi-square,[5] and conclusions
			Observed after mixed[4] pollination	Expected from self-& cross-pollination	
15B	15x17	10	171:3	158,6:15,4	10,1**
20	1x5X	3	152:7	139,7:19,3	8,2**
21	30x5X	5	191:6	163,7:33,3	26,0**
24	13x5X	5	185:6	170,5:20,5	10,7**

[1]See numbered tests Table 19, page 82 .

[2]5X, cross-pollination using pollen from five different clones mixed in approximately equal proportions.

[3]See deviant types Table 10, page 54.

[4]Mixed-pollination using the self and cross pollens mixed in equal proportions by weight.

[5]Chi-square and conclusions testing the null hypothesis of no difference between the observed normal to deviant ratios after mixed- (self and cross)-pollination and those expected from separate self- and cross-pollination. **Significant at 1 percent level.

Selective fertilisation based on deviant progeny

The outcome of selective fertilisation tests was judged using Chi-square applied to the ratios of normal to deviant progeny shown in Table 25 (p. 102). Conclusions were reached in the manner described below.

In Test 15 the observed ratio after mixed-pollination was 171:3 (total 174). Self-pollination resulted in a ratio of 84:18 and there were no deviants among the 91 crosses. The expected ratio in a theoretical mixture of self and cross pollen would be 84:18 for the selfed half plus 102:0 for the crossed half, i.e. 186:18, or 158,6:15,4 (total 174) (Table 30). There was a highly significant difference (Chi-square = 10, P = <01) between this expectation and the observed ratio in the mix of 171:3 and selective fertilisation therefore probably occurred, in favour of the cross pollen.

In Table 30, the observed ratios after mixed-pollination are repeated from Table 25, and those expected from the self and cross are shown converted in the above manner so that their total equals that observed in the mix. Values for Chi-square are also shown in testing the null hypothesis of no difference between the observed and expected ratios.

Differences were highly significant in the four tests of four clones and it is concluded that when self and cross pollens were in competition, the proportion of deviants was reduced significantly below what was expected from the ratios obtained after separate application of the two pollens. There were strong indications that selective fertilisation had occurred in favour of cross pollen in each case.

In the foregoing, the deviant types used were the same as those for which each test was undertaken originally, but in Tests 21 and 24, other deviant types developed unexpectedly and it seems clear from inspection of the figures in Table 25 (p. 102), that in regard to these types also, (Type 11 in Test 21 and Types 11 and 7 in Test 24), a similar favouring of cross pollen is indicated since, in each case, only a single deviant was observed in the mix.

Selective fertilisation based on seeds per capsule

For this purpose, Tests 13A to 17A were used. These were done concurrently with Tests 13B to 17B using the same pollen collection (p. 83), but the pollination was done to flowers at comparable stages, so that seed yields could be compared.

The intention was to observe seed yields per capsule after mixed- (self and cross)-pollination, to note the resulting values in relation to yields after separate self-

Table 31

Assessment of selective fertilisation based on numbers of seeds per capsule after mixed-pollination in relation to self-pollination and cross-pollination

Test[1]	Cross[2]	Seeds per capsule	Differ-[3] ence	Test[1]	Cross[2]	Seeds per capsule	Differ-[3] ence
15A	15x15	10,3		17A	10x10	0,5	
	15xM	33,2	22,9		10xM	3,8	3,3
	15x17	53,0	19,8		10x17	12,2	8,4
20	1x1	8,7		16A	17x17	0,9	
	1xM	20,0	11,3		17xM	22,7	21,8
	1x5X	29,4	9,4		17x15	37,7	15,0
21	30x30	10,1		14A	19x19	1,0	
	30xM	23,3	13,2		19xM	8,9	7,9
	30x5X	31,1	7,8		19x6	32,7	23,8
24	13x13	26,2		23	24x24	1,8	
	13xM	28,4	2,2		24xM	19,4	17,6
	13x5X	56,0	27,6		24x5X	24,2	4,8

[1]See numbered tests Table 19, page 82.

[2]5X, cross-pollination using pollen from five different clones mixed in approximately equal proportions; M, mixed-pollination using the self and cross pollens mixed in equal proportions by weight.

[3]In each test, the upper line shows the difference in numbers of seeds per capsule between mixed- and self-pollination and the lower line the difference between the mixed- and cross-pollination

pollination and cross-pollination, and to see how these results compare with those from phenotypic frequencies of deviant types.

It is to be noted that the seed yields include all germinations, while the deviants represent plants remaining after random discard (p. 85) and surviving for planting (p. 101).

The likelihood of selective fertilisation has already been demonstrated in Tests 15B, 20, 21 and 24 (Table 30, p. 114), and numbers of seeds per capsule for the last three of these, and for Test 15A are shown on the left side of Table 31. The four clones on the right have not been so tested. For the former, since ratios among the seedlings indicated that cross pollen had been favoured, one would expect the numbers of seeds per capsule from mixed-pollination to be closer to those from crossing than to those from selfing. This was the case in three tests, but the differences were mainly quite small and in the fourth test, number 24, the mix was much closer to the self than to the cross, so that the self pollen appeared to have been favoured.

Because of this inconsistency there would be lack of confidence in using results obtained by this means in the absence of other bases as a check. This is, if anything, confirmed by the four tests on the right of the table, where the mix was nearer to the self than to the cross in Test 14 of G19 and in Test 17 of G10. Although this may indicate selective fertilisation in favour of self pollen, it seems unlikely that this would apply in the case of a clone with a relative yield as low as 4%, as in G19 (Table 20, p. 87).

Selective fertilisation based on heights

The intention was to compare the progeny from mixed-pollination with those from selfing and crossing combined and to base results on differences in mean height between these two. These results are then compared with those obtained from normal to deviant ratios.

Mean heights of trees in randomised blocks of four tests are recorded on the right side of Table 32, which shows the values for self and cross combined, also for the mix. The observed ratios after self-, cross- and mixed-pollinations (from randomised blocks only) are shown on the left side of the table, also the expected ratios, as calculated from the self and cross. In each of four tests, the observed

Table 32

Assessment of selective fertilisation based on mean heights of progeny from mixed-, self- and cross-pollination, compared with results based on normal to deviant ratios in the same progeny

Test[1]	Treat-[2] ment	Ratios normal : deviant Observed	Expected from self and cross	Chi-square[3] and conclusions	Nr. trees	Mean heights m	t[4] and conclusions
15B	self	28:10	33,9: 5,1	2,9^{NS}	38	1,36	,7^{NS}
	cross	40:0			40		
	mix	38:1			39	1,46	
20	self	46:14	53 : 7	2,0^{NS}	60	2,20	2,4*
	cross	60:0			60		
	mix	57:3			60	2,42	
21	self	44:21	58,7:11,3	6,4*	65	1,81	3,7**
	cross	68:0			68		
	mix	67:3			69	2,21	
24	self	65:25	77,5:12,5	9,3**	88	1,91	5,5**
	cross	90:0			90		
	mix	88:2			90	2,40	

[1]See numbered tests Table 19, page 82.

[2]The mix treatment consists of mixed pollination using the self and cross pollens mixed in equal proportions by weight.

[3]Chi-square and conclusions testing the null hypothesis of no difference between the observed normal to deviant ratios after mixed-pollination and those expected from cross-pollination.

[4]Values for Student's t and conclusions comparing mean heights of mix-pollinated progeny, with those from self-pollination and cross-pollination combined.

^{NS}Not significant. * Significant at 5 percent level.

**Significant at 1 percent level.

proportions of deviant plants in the mix was less than the expected proportions of these plants, but although differences were significant in Tests 21 and 24, they were not so in Tests 15 and 20. These results differ from those recorded in Table 30 (which included all progeny), where all differences were highly significant and this is attributed, in part at least, to the lower numbers of plants in Table 32. On combining Tests 15 and 20, differences were significant (Chi-square = 5,4 and P = <,05).

The mean heights gave the same indication as the ratios. In each test, the mean heights from mixed-pollination were better than the mean of the self and cross combined, but although differences were significant in the three Tests 20, 21 and 24, they were not significant in the fourth test, number 15.

Indications from mean heights were mostly comparable with those from ratios. Thus, from the few tests done, assuming that the deviant types give reliable indications, mean height could probably be used to judge whether or not selective fertilisation had occurred.

8.7 Summary and conclusions

Out of 45 clones which were tested by self-pollination, 44 were found to be self fertile; selfed seed yield varied widely between clones and was up to 24 seeds per capsule. A similar range in self-fertility in different clones was observed when selfed seed yield was related to the yield after crossing. When flowers had been control pollinated at comparable stages of development, the yield from selfing varied from 2% to 47% of that from crossing in 22 tests of 11 clones.

This subject was followed up by using genetic markers to estimate natural selfing. The markers available were subject to certain limitations and estimates were made on the basis of the numbers of seedlings which survived long enough to be classified. Results were commonly near the 30% mark and varied from 10% to 38% in seven tests of five clones, done during the main flowering season. For a clone which gave 27% and 38% selfing in two tests during the season, a figure of 83% was obtained for out of season flowering. Most of these results were regarded as maxima, since carriers, other than the clone under test, were often identified within the area concerned.

A test was also made of the suitability of mean tree heights for estimating selfing and results were found to be comparable with the above in four cases out of six.

The reduced seed yield after selfing compared with crossing represented one of the most consistent inbreeding effects and, apart from the development of abnormal types among the selfed progeny, the other main effects were reduced mean height and, to some extent, poorer stem straightness. The effects on percent fruit set, survival and percent capsules having seed were mostly inconsistent.

In 12 replicated tests of ten clones, there were significant differences between self-pollinated and cross-pollinated progeny in mean height, the self being depressed in relation to the cross by 8% to 49% at age 11 to 18 months after sowing the seed. This tendency was still in evidence at age three years, when there was a corresponding inbreeding depression in diameter at breast height. At this stage also, scores for bole straightness were significantly less after selfing than after crossing in two tests out of three.

Comparing the results of open-pollination with those from crossing, in 11 out of the above 12 tests, the depression in mean height at age 11 to 18 months was from 2% to 26% and at three years of age, combining scores for height and bole straightness in three tests, the mean scores after open pollination were depressed by 8%, 11% and 13%. The differences were significant in each of the latter and these figures are taken to represent the overall degrade resulting from the amount of selfing occurring after open-pollination compared with the results of the cross-pollination done in the tests.

The indicated amount of natural selfing combined with the occurrence of out of season flowering and the inbreeding effects observed on tree vigour and form, make it desirable to consider what steps should be taken to minimise the effects of these on the open-pollinated progeny of a seed orchard. One possibility is to reject the more self-fertile clones, as identified by one of the three procedures referred to above, i.e. by either taking account of the numbers of seeds per capsule after controlled self-pollination on its own, or by comparing the results with those from cross-pollination, or by making use of estimates of the amount of natural selfing.

Culling of suspected deviant types of seedlings in the nursery would of course be a normal operation which would have some effect in certain cases, while hand-pollination in the seed orchard would, at least theoretically, give a higher proportion of crossing. The latter statement is made in view of the results obtained from an examination of selective fertilisation.

The use of genetic markers showed that there were strong indications that selective fertilisation occurred in favour of the cross pollen when self and cross pollens were in mixture. It appeared that mean heights of planted trees could be used as a second means of assessment, but that earlier measurements, of germination time and epicotyl length, were unsuitable. The same conclusion was reached regarding numbers of seeds per capsule as a means of assessment.

Concerning other barriers to selfing, there was no consistent difference between the speed of germination of pollen on the stigma after self-pollination compared with cross-pollination. Other possibilities, such as inhibition of growth of pollen tubes in the style, were not examined.

Chapter 9

DISCUSSION AND CONCLUSIONS

Clonal seed orchards have been established at Zomerkomst by grafting from selected *E. grandis* trees, with the object of producing bulk seed for afforestation with improved types, especially in regard to timber quality. As this object may be affected adversely by inbreeding, it is necessary to ascertain which of the selected clones are self-fertile, to what extent natural selfing is likely to occur and the nature of any inbreeding depression. It may become necessary to find some means of controlling selfing, while any natural barriers to selfing which exist form a related study, and various investigations in connection with flowering, fruits, seeds and seedlings are considered basic.

Many of the above questions were investigated by means of controlled pollinations, mostly done in a tree bank situated at low altitude, though some were done in a seed orchard at a higher altitude where the rainfall is also higher.

Onset of flowering occurs at two to three years of age in self- and cross-pollinated progeny, also in grafted plants and cuttings. The period required for flower buds to reach anthesis is about four months in the tree bank, but rather longer in the seed orchard, so that bud development is slower there, probably because of the lower temperatures which are experienced.

Regarding the time required for ripening of the fruit, Pryor (1951 and 1956), referring to the genus as a whole, gives periods of eight to 12 months, but this is known to vary widely with species. Locally, *E. grandis* fruits were considered ripe some five months after anthesis in the tree bank and seven months after anthesis in the seed orchard, although the seed was viable earlier. Since there was so much variation with site, separate tests of maturity would be needed for each new locality, although maturity as indicated by browning of the capsules and dark seed colour, could be expected to apply at any site; these colour changes provide a line on capsule maturity for those cases where the date of anthesis is unknown.

It is concluded from the foregoing that, under local conditions, a single generation may be completed within four years, which is a short cycle for breeding work with tree species.

The season of flowering also varies with site, since Blakely (1955) gives June to August for *E. grandis* in Australia, while the main flowering season is February

and March in the local tree bank and April to June in the seed orchard, although a considerable amount of out of season flowering occurs, especially at the latter site. This may be because the species is too far outside its natural range in the seed orchard, which is at an altitude of some 1 300 metres (and latitude 24 degrees South), but in any case, the unseasonal flowering is an undesirable feature in seed orchards, especially in that it results in more selfed seed than usual.

The unit inflorescence is a dichasium, commonly referred to as an umbel. From 1966 to 1972 it was observed to be typically seven-flowered, but since seven to 14 flowers per umbel were observed in 1973, this feature seems to be subject to seasonal variation. It is also noted that Blakely (1955) states that the umbel is three-flowered to ten-flowerd.

As described by Carr & Carr (1959a) for the seven-flowered condition, the umbels are at first enclosed in an involucre of six or more bracts. The receptacle of the flower bears on its rim two opercula representing the calyx and corolla, and a ring of numerous inflexed stamens, and it encloses the inferior ovary.

Workers outside Australia are normally in a relatively poor position to appreciate the taxonomy of the genus as a whole, but recent advances which have been made are helpful. Various floral and other characters, not previously taken into account, are used by Pryor & Johnson (1971). They provide evidence for classification, by which the genus is divided into eight subgenera, *E. grandis* being placed in the subgenus *Symphyomyrtus.*

The material examined locally conforms to descriptions by Johnson (1972) for some of the subdivisions of *Symphyomyrtus* in that the flowering shoot ends in an active vegetative bud, there are two independently shed opercula, and the cotyledons are emarginate in shape. The relative timing of initiation of the two perianth whorls also conforms with that described by Pryor & Knox (1971) for related species. The subsequent initiation of the staminal primordia at the base of the petals is also in conformity, but the more or less simultaneous development of a structure which later forms the style, appears precocious compared with illustrations by Pryor & Knox, and while the anthers conform to the illustrations by Blakely (1955) for the section *Macrantherae* and the subsection *Longiores,* they are rather more obovate in shape.

Other points on which the material examined differs from the published records are that while Johnson (1972) records the ovules as being hemitropous, those ob-

served vary from hemitropous to anatropous. In addition, the ovules and those ovulodes which are mixed with them are not arranged on the placenta in as regular a manner as might be expected from the illustrations by Carr & Carr (1962b).

Other aspects of floral morphology which are worth noting in connection with the taxonomy of the species are referred to below, where they are related to observations on flower phenology. Some of the latter are mentioned in connection with their practical significance in controlled pollination operations.

The shedding of the bracts and of the two opercula divides the bud development into three distinct phases, each some four to six weeks in duration and totalling about four months. By the time the floral buds had become recognisable as such, the sepaline operculum had already developed and the petaline primordia had been initiated, followed some ten days later by the initiation of the staminal primordia. The microspore tetrads are tetrahedral and are formed while two opercula are still present, but before the outer operculum becomes discoloured prior to being shed.

The ovary contains numerous ovules and infertile ovulodes, which are initiated as the time for bract shed approaches. Two types of ovulodes are recognised; one occurs round the top of the placental column and gives rise to the "elongated chaff" of the mature fruit. The other type occurs in the lower half of the ovary, where they sometimes form the inner two of four vertical rows down the placenta, with a row of ovules flanking them on either side. The latter type of ovulode gives rise to the "truncate chaff".

Two integuments grow round the nucellus of the ovules some four weeks after their initiation, at about the same time as the formation of the microspore tetrads. Initially, as in the case of the ovules, the truncate ovulodes also develop two integuments and the same feature was occasionally seen in some of the elongated ovulodes. Later, in both of these structures, only a single, strongly-stainable outer layer of cells remains clearly recognisable.

A special feature of the ovary is that while the placentation is axile at the base, typically with five locules, the partitions between the locules are incomplete at the top of the ovary, where the elongated ovulodes are arranged in a manner similar to that of free central placentation, except that the septa extend upwards to the top of the ovary.

The main features taken into account in connection with controlled pollination are the development of the flower bud, pollen shed and stigma receptivity.

Yellowing of the inner operculum prior to anthesis is a useful indication that the buds are at a suitable stage for emasculation.

There are several possible sources of contamination during the controlled pollination process, one of these being the presence of pollen on the anthers of mature buds, which would result in self-pollination during the emasculation process. But although this pollen was found capable of giving self-fertilised seed when used on receptive stigmas, very little seed usually resulted from using it to pollinate mature buds, and there is therefore not as much danger of contamination from this source as might be expected. Nevertheless, in testing the efficiency of isolation during emasculation and bagging of mature buds, ten seeds were derived from 648 capsules and it was concluded that all possible precautions should be taken to guard against contamination.

With regard to the role of stigma receptivity in controlled pollination, some seed was produced after cross-pollination done at and even before anthesis, although maximum seed yield was not obtained until some five days later. This was before the stigmas had developed a distinctly swollen and sticky condition. After this point was reached there were diminishing yields, normally up to the seventh day or longer. This provides a guide to the best time for controlled pollination, both according to the number of days from anthesis as well as from the appearance of the stigma.

There were appreciable day to day changes in receptivity, so that in doing tests comparing numbers of seeds per capsule, all pollinations need to be done at comparable flower stages.

Although seed yields gave the impression that the stigmas were receptive at about the time of anthesis, this was found to result from the fact that the pollen is capable of remaining on the stigma for a few days before germinating. There was some germination (probably *in vitro*) at anthesis, but the main germination on the stigma did not commence until two or more days later, depending on the clone, and this is taken to be the beginning of the true receptive stage.

In any one flower, the end of the main pollen shed coincides with the beginning of true receptivity (to cross pollen) in some clones, and this is taken to indicate a protandrous condition which, however, is regarded as being partial rather than absolute.

It was found that wind, or the dropping of pollen, can result in some (self)-fertilisation, since some seed was produced from buds which had been bagged and left undisturbed. However, the amounts of seed were small and, as is the case elsewhere for the genus as a whole, insects appear to be the main pollinators. Of the many species seen visiting flowers, the honey bee seems to be the most effective. This is mainly because bees were rarely seen to visit flowers from which the pollen had been shed, and the yield of seed from emasculated buds was usually much reduced when compared with seed obtained from untreated buds.

In view of the remarks by Larsen (1965) and as a result of local experience, no steps were taken for the breaking of dormancy when germinating seed. Larsen also states that *Eucalyptus* seed can be kept viable for many years and this was confirmed for *E. grandis* as a result of a test done over a (relatively short) period of seven years.

The presence of chaff mixed with the seed presents special problems and some means of improving purity is desirable. In *E. grandis,* in most cases the seed may be distinguished from the chaff on the basis of differences in colour and shape. Larsen (1965) states that seed and chaff of the species differ in regard to size, but this view could not be confirmed here and no use could be made of differences in size for separating the two. Although it was not possible to obtain pure seed by sieving, by doing this and retaining all particles of 500 µm and over in size, the total weight was reduced by over 50%, and 84% of the weight of seed was retained. This fraction also contained seed with the shortest germination time and gave rise to seedlings with the best survival and height growth. A sieving procedure before doing germination tests and despatching the seed for sale could thus be followed with some advantage.

A weight of about 10 g of seed and chaff was obtained from 500 capsules and the proportion of seed to chaff by weight was about one to five.

There seems to be some doubt about the extent to which parthenocarpic fruit is set within the genus (Pryor, 1951; Penfold & Willis, 1961). After emasculating and bagging *E. grandis* flower buds, some fruit was produced from 12 of the 18 clones tested, the percent fruit set varying from 1% to 92%. Most clones were therefore capable of setting some parthenocarpic fruit, although the ability to do this varied widely.

In some clones, losses of capsules were liable to occur as a result of abscission following lack of fertilisation after certain controlled pollination treatments, and such

losses were liable to give rise to inaccuracies in any results based on numbers of seeds per capsule.

There were also other losses, attributable to mechanical damage, and in recording controlled pollination operations, it was considered best to keep a record of these in an attempt to take some account of them in interpreting results. Some clones were found to be particularly susceptible, since even handling during emasculation seemed to cause damage to the pedicel resulting in the loss of all flowers. There were thus some clones which could not be used for controlled pollination if emasculation was involved.

To test the incidence of self-fertility among the various clones, flower buds of 45 clones were bagged and self-pollinated, some more than once, in the course of four seasons. In one clone which was not tested a second time, no self-fertilised seed was produced, but all others yielded some seed on at least one occasion. Yields varied widely from nought to 24 seeds per capsule and the seedlings were mostly viable. It is therefore concluded that while there is great inter-clonal variation in the degree of self-fertility, at least 44 of the 45 clones tested are to some extent self-fertile. This result is contrary to reports by Krug & Alves (1949) and Wright (1962), who record *E. grandis* as being self-sterile. This difference may possibly be associated with variation between geographical areas (Hagman, 1967).

In a search for suitable markers to be used in assessments of natural selfing, among the progeny of 44 clones examined, 15 different types of abnormalities were recognised in 25 clones. Ten were classed as detrimental and eight of these did not develop until near or after planting time. No irregularities of any consequence were seen during germination and no progeny showed distinct and consistent chlorophyll deficiencies, most abnormalities being of leaf or stem form.

The more clearly defined of these deviant types were used as markers in assessing the amount of natural selfing in open-pollinated progeny. This was commonly round the 30% mark and varied from 10% to 38% in seven tests of five clones, done between 1968 and 1972 during the main flowering season. For the clone which gave 38% selfing, a figure of 83% was obtained for out of season flowering. The results for seasonal flowering are comparable with the 8% to 28% obtained for *E. regnans* (Eldridge, 1970), also with the 77,2% "cross-pollination" reported for *E. alba* by Krug & Alves (1949).

In interpreting these results, account must be taken of the limitations to which such estimates are subject. As mentioned by Sorensen (1967) and by Franklin (1971b),

some of these limitations are poor definition of markers, limited numbers in some selfed families and the effects of embryo mortality. Further, as stated by Fryxell (1957) for entomophilous species, variation may be expected from one season to another, since results would be affected by the types and numbers of insects present and their activity in relation to the numbers of flowers and weather conditions.

In the present case, nothing is known of embryo mortality and since many markers develop late, information about the effects of subsequent inviability is incomplete and results are of the number of surviving seedlings, and not of the number of actual self-fertilisations. The markers also had the disadvantage that they were not always well defined, the numbers in some selfed families were low and there was sometimes inconsistency in that deviant progeny were formed in some seasons but not in others.

All clones in the area were tested by selfing, thus giving an idea of the number of carriers present, and since several of these were identified in clones other than those under test, most of the estimates were regarded as maxima.

The general conclusion therefore is that, as stated by Pryor (1957 and 1961) for the genus as a whole, the clones tested are mainly outbreeding. This is confirmed by the consistent inbreeding effect on height referred to later, which is typical of outbreeding species (Williams, 1964).

Since suitable markers were found in relatively few clones, other means of assessing natural selfing were considered, but of these, only mean tree heights gave promise. Measurements after open-pollination (O) were intermediate between those from self-pollination (S) and cross-pollination (X) in 11 out of 12 replicated tests. Applying the mean heights in the formula (X-O/X-S) 100 (Franklin, 1971a), results were within 10% of those from deviant seedlings in four out of six tests. The closer conformity was obtained when mixed cross pollens were applied rather than single cross pollens.

While the heights of planted trees may usually serve as a guide to percent selfing, heights of young seedlings in the nursery could not be used for this purpose. This is because of the effect, on early vigour, of variation in number of seeds per capsule (p. 131).

If appreciable amounts of self-fertilisation are found to occur in nature, it then becomes all the more desirable to find out what inbreeding effects there may be.

Apart from reduced seed yield and the production of deviant seedlings and young transplants, the main effects were on mean height and in some cases on bole straightness. The former confirms Pryor's (1961) statement that marked depression of growth can occur following inbreeding in eucalypts. There were no consistent effects on fruit set, survival, or the percent capsules having seed.

In 12 replicated tests of ten clones, there were significant differences between self-pollinated and cross-pollinated progeny in mean height, the self being depressed in relation to the cross by 8% to 49% at age 11 to 18 months after sowing the seed. This tendency was still in evidence at age three years, when there was also a corresponding inbreeding depression in diameter at breast height. At this stage also, scores for bole straightness were significantly less after selfing than after crossing in two tests out of three.

Comparing the results of open-pollination with those from crossing, in 11 out of the above 12 tests, the depression in mean height at age 11 to 18 months was from 2% to 26% and at three years of age, combining scores for height and bole straightness in three tests, the mean scores after open-pollination were depressed by 8%, 11% and 13%. In the latter, the differences were significant in each case and these figures are taken to represent the overall degrade resulting from the amount of selfing occurring in open-pollinated progeny, compared with the results of the cross-pollination done in the tests.

Concerning the natural barriers which may exist to selfing, two factors gave the impression that there is a preponderance of self-pollination. Pollen may be seen to be deposited on a stigma as the stamens unfold and as the anthers brush past it. Also, although bees must bring some cross pollen from the hive, they are most persistent in foraging on the same ramet for long periods. When this apparent prevalance of self-pollination is considered in relation to the relatively low indications for self-fertilisation, it is evident that some barriers are in operation.

Although nearly all clones are self-fertile in varying degrees after pollination done at or near maximum receptivity, seed yield from self-pollination done during the first two days after anthesis was mostly negligible. An unspecified barrier was therefore in operation at this stage, which would limit selfing within flowers, as the pollen is being shed. However, there still remains selfing between flowers, which could result in appreciable amounts of seed if pollen were transferred from a newly opened flower to the stigma of another flower at maximum receptivity. But as far

as bees are concerned, this would not happen often, since bees were rarely seen on flowers which had reached a receptive stage.

It was found that pollen is capable of remaining viable outdoors for about four days and since (cross) pollen placed on a stigma at anthesis began to germinate two to four days later, little or no part would be played by loss of pollen viability during this period. But there was a loss in the actual number of grains remaining on the stigma, since it was noted that when pollen was placed on stigmas at a series of stages of receptivity and a count made up to 17 hours later, there was a regular increase in the number of grains which were retained, from 282 per stigma pollinated at or near anthesis, to 1183 on stigmas pollinated four days later. Therefore pollen is easily lost from as yet unreceptive stigmas and this, combined with a considerable degree of protandry, is seen as limiting the amount of pollen remaining on the stigma for possible germination later.

While the above factors would probably contribute in limiting the amount of effective self-pollination within and between flowers during the first two days, the above mentioned unspecified barrier at this stage is probably overrriding. There is also a barrier having effect throughout later flower stages, since seed yield from manipulated selfing was invariably less than that from crossing.

When all pollinations were done at comparable stages of receptivity, taking seed yield per capsule from cross-pollination as 100%, the relative yield after selfing, or "self-ability" (Bingham & Squillace, 1955), worked out at between 2% and 47% in 22 tests of 11 clones. Therefore, as in other genera (Taft, 1966 and others), a wide range in self-fertility is indicated in different clones.

The above figures for relative yield differ from a preliminary report for *E. grandis* by Burgess (1973), who found no difference between cross-fertilised and self-fertilised progeny in numbers of seeds per capsule.

Regarding the cause of reduced seed yield after selfing, no consistent differences were observed between the germination of pollen on the stigma after selfing compared with crossing. Pollen tube growth was not examined, and incompatibility in the style therefore remains a possibility. However, there was no direct evidence of this and reduced seed yield after selfing is referred to tentatively as being due to self-infertility, as has been found to be the case for many other tree species.

Selective fertilisation, having effect at an unspecified stage in the reproductive cycle (Squillace & Bingham, 1958), was investigated using various methods. An attempt

was first made to do this according to the relative values for seed germination time and epicotyl length in self-, mixed- and cross-pollinated progeny (Barnes *et al.*, 1962), but this method was discarded because any differences between the self and the cross were masked by the effect which the number of seeds in a capsule had on these variables. Germination time was longer when there were large numbers of seeds per capsule (as after cross-pollination), compared with capsules having few seeds, as in selfing. The results for epicotyl length followed a similar trend.

Using genetic markers, when self and cross pollens were mixed and applied in equal amounts by weight, in each of four clones, the proportion of deviants which resulted was significantly lower than that expected from application of the two pollens separately. There were therefore strong indications that when mixed- (self and cross)-pollination was done, selective fertilisation occurred in favour of the cross pollen.

Numbers of seeds per capsule did not appear likely to give a reliable means of assessing selective fertilisation, but indications from mean tree heights were, on the other hand, similar to those from deviant seedlings. It is therefore concluded that heights could provide a second basis for testing whether selective fertilisation had occurred, although further investigation of this point would be desirable.

The general conclusion reached is that the clones examined are entomophilous cross breeders, but that in spite of certain natural barriers to selfing, some self-fertilised seedlings are liable to be present in the natural progeny. The number of such seedlings is likely to be very high in cases where they are derived from out of season flowering, but otherwise, many of them would normally be removed in the first thinnings. Nevertheless, they are subject to inbreeding depression in vigour and form and their production is undesirable for an undertaking devoted entirely to tree improvement.

It is necessary not only to know about such features of the reproductive system of the species, but also to take appropriate action.

Although there are other possibilities, the three possible courses of action most closely related to the present work are culling of all suspect seedlings in the nursery, rejection of the more self-fertile clones, and hand pollination in the seed orchard.

Although many of the deviant types seen do not develop clearly till later, initial symptoms in the nursery include dwarfing, red foliage, sparse foliage, small leaves, or any deformities of the leaves or stems. While it may be routine practice to cull such

plants, the concern should be rather to ensure that the seed which gives rise to them is not put up for sale, and for this, one must turn to the other two possibilities.

To reject the more self-fertile clones, these may be identified according to seed yields from self-pollination, or from self-pollination compared with cross-pollination, the selfing and crossing being done at comparable stages of receptivity. These observations would provide a rough comparison between clones on which to base decisions about their possible rejection, but would not take account of some natural barriers; a high selfed seed yield under these conditions would not necessarily mean a high level of selfing in nature. To estimate the amount of natural selfing would be a better method; this could be either based on markers, or on the mean heights of trees from open-pollination compared with self- and cross-pollination.

Whatever course is followed, some test of self-fertility and of inbreeding depression should be done for each clone as it comes into production.

As regards hand pollination, theoretically at least, this could be done on suspect clones, using a mixture of pollen from all the clones in the seed orchard; emasculation would be mostly unnecessary. Because of the probable effect of selective fertilisation, especially with the preponderance of cross pollen which would be applied, very little self-fertilisation would be expected. The risk of this would be even less if pollination was done at early flower stages within two days after anthesis.

SUMMARY

Phenological and morphological studies of the flower were done at Zomerkomst Forest Research Station, in order to obtain a clearer understanding of floral biology. The breeding system and related subjects could then be investigated, with a view to ascertaining the amount of natural selfing, the extent of inbreeding depression and their possible effects on the production of improved seed.

The first flowers appear on trees aged two to three years. The flowering season varies widely with altitude; it is mainly from February to June, although some flowering is liable to occur throughout the year.

The flowering shoot ends in an active vegetative bud and consists of up to 14 "umbels", each of which is typically seven-flowered and is at first enclosed in six or more bracts. The receptacle of the flower bud bears on its rim two independently shed opercula and a ring of numerous inflexed stamens and it encloses the ovary.

By the time the floral buds had become recognisable as such, the sepaline operculum had already developed and the petal primordia had been initiated at their base. Staminal primordia are formed some ten days later and tetrad formation occurs before the outer operculum is shed.

The style is initiated at about the same time as the staminal primordia. The ovary contains numerous ovules and infertile ovulodes, which are initiated as the time for bract shed approaches. There are two types of ovulodes, which give rise to two types of chaff in the mature fruit. The ovules are anatropous to hemitropous and have two integuments, each two cell layers in thickness. These develop at about the same time that the microspore tetrads are formed.

The ovary is inferior, typically with five locules and axile placentation, except at the top where the partitions between the locules are incomplete.

The flowers are regarded as being protandrous, or at least partially so, and mainly insect pollinated, especially by honey bees. Pollen shed was mostly complete by the second day after anthesis, and some seed was obtained after cross-pollination done at anthesis, although germination of pollen on the stigma did not usually begin until two or more days later. Maximum seed yield was obtained from pollination done on the fifth day after anthesis, before the stigma developed a distinctly swollen appearance. The stigma remained receptive up to the seventh day after anthesis or later, and there were pronounced day to day changes in receptivity.

The fruit is a capsule which is mature in five to seven months, depending on the site. In most clones tested, some fruit was set parthenocarpically. Most seeds may be separated from the chaff on the basis of colour and shape, but not size, although by passing through a sieve of gauge 500 µm, most of the seed was retained. Such seed was the best as regards early vigour and survival and the sieving gave increased purity.

In tests of self-fertility, in which all pollinations were done at comparable stages of receptivity, the seed yield from self-pollinated flowers varied in different clones from 2% to 47% of that from cross-pollination.

Out of 45 clones which were self-pollinated, 44 yielded some seed, varying in number up to 24 seeds per capsule. Among the progeny, 15 different types of abnormality were found and four of these were used as the main means of assessing natural selfing. This was commonly about 30%, but was 83% in one test of out of season flowering. Mean tree heights gave promise as a possible alternative basis to be used in estimating selfing.

Although selfing was rather high in some clones, the species was mainly outbreeding. This conclusion is supported by the consistent inbreeding effect on mean tree heights, which was observed at age 11 to 18 months after sowing the seed. This varied in different clones from 8% to 49% comparing self-pollinated progeny with that from crossing. Comparing the result of open-pollination with that from crossing, apart from one case, the depression varied from 2% to 26%. There was also some inbreeding effect on bole straightness and when this effect was added to that on height (as represented by relative dominance), scores for progeny from open-pollination were depressed in relation to those from cross-pollination by 8% to 13% in three tests at age three years.

Bees were not often seen on flowers which had been open for more than two days, and this fact is seen as a natural barrier to selfing, in that when they are most receptive to self pollen, stigmas are not often pollinated by bees. In addition, pollen is easily lost from unreceptive stigmas and this, combined with a considerable degree of protandry, is seen as controlling the amount of pollen remaining on the stigma for possible germination later. But there was an overriding barrier to selfing, in that there were invariably fewer self-fertilised seeds than cross fertilised seeds after hand pollination and there were strong indications that when mixed- (self- and cross)-pollination was done, selective fertilisation occurred in favour of the cross pollen.

It is necessary not only to know about such features of the reproductive system, but also to take appropriate action. Some self-fertilised seedlings are liable to be present in the natural progeny and these are subject to inbreeding depression in height and form. Some of these seedlings may be removed by culling in the nursery, but it would still be necessary to do testing and rejection of the more self-fertile clones. Hand-pollination in the seed orchard is another possible way of reducing selfing and this would be at least theoretically effective.

OPSOMMING

Fenologiese en morfologiese studies van die blomme van *Eucalyptus grandis* is op Zomerkomst Navorsingstasie gedoen om daardeur 'n beter begrip van die biologie van die blom te verkry. 'n Teelprogram met die oog op die bepaling van die mate van selfbestuiwing, die nadelige gevolge van inteling en die invloed daarvan op produksie van verbeterde saad, kon daarna ondersoek word.

Die bome begin na twee tot drie jaar blom. Die blomperiode varieer aansienlik namate die hoogte bo seespieël varieer, maar strek gewoonlik van Februarie tot Junie, alhoewel blomme sporadies dwarsdeur die jaar voorkom.

Takkies met bloeiwyses behou steeds 'n aktiewe vegatiewe groeipunt en dra tot 14 umbellas, elk kenmerkend met sewe blomme. Aanvanklik is die bloeiwyse (umbella) deur ses of meer skubblare bedek. Die blomme besit elk twee operkulums wat onafhanklik van mekaar afgestoot word en 'n ring talryke ingevoude meeldrade. Bogenoemde organe kom op die torus wat die vrugbeginsel omring, voor.

Teen die tyd dat die blomknoppe sigbaar word het die operkulum wat die kelk verteenwoordig, reeds ontwikkel en die kroonblaarprimordium wat die binneste operkulum vorm, aan die basis daarvan geïnisieer. Meeldraadprimordiums word ongeveer 10 dae later gevorm terwyl tetrades gevorm word voordat die buitenste operkulum afgestoot word.

Die style word min of meer gelyktydig met die meeldraadprimordiums gevorm. Die vrugbeginsel bevat talryke saadknoppe wat gevorm word teen die tyd dat die skubblare afgestoot word. Twee tipes abortiewe saadknoppe ("ovulodes") wat aan twee tipes kaf in die volwasse vrug oorsprong gee, kom voor. Die saadknoppe is anatroop tot hemitroop met twee integumente, elk twee sellae dik. Hulle ontwikkel ongeveer teen die tyd dat mikrospoortetrades gevorm word.

Die vrugbeginsel is onderstandig, met vyf vrughokke en 'n aksiale plasentasie, alhoewel die tussenskotte in die bo-punt onvolledig is.

Dié blomme word as protandries of ten minste gedeeltelik so beskou en is hoofsaaklik insekbestuif; hoofsaaklik deur heuningbye. Die vrystelling van stuifmeel is ongeveer na die tweede dag na antese, afgehandel. Saad is wel verkry van kruisbestuiwings wat tydens antese gedoen is, alhoewel die ontkieming van stuifmeel op die stempel gewoonlik eers op die tweede dag na antese of daarna plaasvind. Die maksimum saadopbrengs is verkry van bestuiwings wat op die vyfde dag na antese, dit wil sê, voor-

dat die stempel die opvallende geswolle toestand bereik het, gedoen is. In die eksperimente het die stempel tot op die sewende dag, of selfs langer, na antese ontvanklik gebly en daar het van dag-tot-dag aansienlike variasies in die ontvanklikheid van die stempel voorgekom.

Die vrug is 'n kapsule wat, afhangende van die groeiplek, na vyf tot sewe maande volwassenheid bereik. In die meeste klone wat ondersoek is het 'n hoeveelheid vrugte partenokarpies gevorm. Saad kan van die kaf geskei word op grond van kleur en vorm, maar nie volgens grootte nie, alhoewel die meeste saad op 'n 500 µm maas sif agterbly. Hierdie gesifde saad was die beste in sover dit kiemkragtigheid en houvermöe betref en het ook 'n hoër persentasie suiwer saad gelewer.

In proewe oor selfvrugbaarheid waartydens bestuiwing op vergelykbare stadiums van ontvanklikheid van die stempel gedoen is, het die saadopbrengs van selfbestuifde blomme by verskillende klone van 2% tot 47% van dié van kruisbestuifde blomme gevarieer.

Uit die 45 klone wat selfbestuif is het 44 wel 'n hoeveelheid saad wat van 'n paar tot 24 sade per kapsule gevarieer het, gelewer. In die nageslag is 15 verskillende tipes abnormale plante gevind waarvan vier as die belangrikste maatstaf vir die vasstelling van die mate van spontane selfbestuiwing gebruik is. Laasgenoemde was ongeveer 30%, maar 83% in een proef waar van buiteseisoenblomme gebruik gemaak is. Gemiddelde boomhoogtes blyk belowend te wees as 'n moontlike alternatiewe maatstaf vir die skatting van die mate van selfbestuiwing.

Alhoewel selfbestuiwing by sommige klone hoog was, kan aanvaar word dat *E. grandis* kruisbestuiwend is. Hierdie gevolgtrekking word gerugsteun deur die konstante nadelige gevolge van inteling op boomhoogte wat op ouderdomme 11 tot 18 maande nadat die saad gesaai is, waargeneem is. Dit het van 8% tot 49% gevarieer waar nageslagte van selfbestuifde en kruisbestuifde blomme met mekaar vergelyk is. Waar die resultate van oopbestuiwing met dié van kruisbestuiwing vergelyk is, was die onderdrukking op een uitsondering na, 2% tot 26%. Daar is ook 'n mate van nadelige invloed van inteling op reguitheid van die stamme waargeneem en waar hierdie invloed by dié van boomhoogte gevoeg is (soos verteenwoordig deur relatiewe dominansie) is die nageslag van oopbestuifde blomme in verhouding met die nageslag van kruisbestuifde blomme by drie proewe, op 'n ouderdom van drie jaar, met 8% tot 13% onderdruk.

Heuningbye is nie dikwels op blomme wat vir meer as twee dae oop was, waargeneem nie en hierdie feit word beskou as 'n natuurlike struikelblok vir selfbestui-

wing in soverre dat wanneer stempels die meeste ontvanklik is vir dieselfde boom se stuifmeel, hulle nie dikwels deur bye bestuif word nie. Verder gaan stuifmeel op nie-ontvanklike stempels maklik verlore. Genoemde feit, tesame met die aansienlike mate van protandrie, beperk die hoeveelheid stuifmeel wat op die stempel agterbly vir moontlike ontkieming op 'n later stadium. Daar was egter deurgaans 'n hindernis ten opsigte van selfbestuiwing aangesien die getal sade van selfbevrugte saadknoppe na handbestuiwing aansienlik laer was as dié van kruisbevrugte saadknoppe. Duidelike aanduidings dat selektiewe bevrugting in die guns van kruisbestuiwing plaasgevind het in gevalle waar gemengde stuifmeel, (die plant se eie stuifmeel gemeng met 'n ander plant se stuifmeel) gebruik is, is gevind.

Dit is noodsaaklik dat daar nie alleen kennis geneem word van bogenoemde feite nie, maar dat daar ook tot gepaste aksie in toekomstige teelprogramme oorgegaan moet word. Kiemplante van selfbestuifde blomme wat onderhewig is aan onderdrukking van hoogte en swak vorm weens inteling, kan in die natuurlike nageslag voorkom. 'n Aantal van hierdie kiemplante mag wel in die kwekery verwyder word maar dit sal steeds nodig wees vir die toetsing en uitskakeling van klone wat self-vrugbaar is.

Handbestuiwing in die saadboorde is 'n ander moontlike manier om selfbestuiwing te verminder en dit sal ten minste teoreties effektief wees.

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Appendix 1

Stigma receptivity on the basis of number of seeds per capsule: numbers of flowers, capsules and seeds, and appearance of the stigma, for the first ten days after anthesis

Test Cross Site[1]	Bag	Day[2]	Numbers of flowers pollinated	capsules reaped	capsules used/ with seed[3]	seeds	Stigma[4]		
1/66	159	1	7 –	3	3/	5	pointed	dark	dry
15x17	156	2	8	7	7/	153	round		
T.B.	151	3	14	12	12/	406			
	148	6	19	17	17/	208	swollen	light	sticky
	142	7	5	5	5/	43			
	137	8	13	10	10/	6	. .	white	. .
1/66	157	1	10	9	9/	3			
15x15	154	2	8	5	5/	33			
T.B.	153	3	8	6	6/	43			
	150	4	16	16	16	70			
	147	6	11	10	10/	9	as above		
	144	7							
	138	8	9	9	9/	0			
2/67	44	0	8 –	2	2/1	9	pointed	dark	dry
17x6	43	1	8 –	0					
T.B.	42	2	8 –	4	4/2	3			
	40	3	7 –	2	2/1	1			
	39	4	5	4	4/4	62			
	38	5	12	8	8/8	117	swollen	light	sticky
	35	6	3	3	3/1	4			
	30	7	7 –	0					
	28	8	7 –	0				white	
3/67	61	0	10	9	9/6	28	pointed	dark	dry
6x17	59	1	8	7	7/5	43			
T.B.	57	2	12	7	7/7	42		light	
	55	3	7 –	2	2/2	22			
	51	4	7	4	4/0	0	round		
	48	5	10	7	7/7	251			sticky
	47	6	8	5	5/4	58	swollen		
	46	7	9 –	0					
	45	8	9 –	0					
4/67	167	0							
10x17	164	1	7	7	7/3	10	pointed	dark	
S.O.	156	2	10	6	6/6	105	round		
	150	4							
	144	6	17	16	16/16	637	swollen		sticky
	141	7						white	
5/67	166	0							
15x17	163	1	7	6	6/1	1	pointed	dark	
S.O.	158	2	6	6	6/3	17			
	152	4	13	12	12/8	136			
	148	6					round	light	sticky
	136	8	7	7	7/7	178			

Appendix 1 (continued)

Test Cross site[1]	Bag	Day[2]	flowers pollinated	Numbers of capsules reaped	used/ with seed[3]	seeds	Stigma[4]		
6/67	165	0	3	3	3/3	14			
19x17	162	1	6	5	5/4	22			
S.O.	160	2	4	3	3/2	13			
	154	4	19	19	19/19	464			
	146	6	3	3	3/3	112			
	138	8	4 –	1	1/1	20			
7/68	124	0	12	10	10/3	11	pointed	dark	dry
6x19	120	1	19	15	15/4	25			
T.B.	119	2	5	4	4/1	5	round		
	113	3							
	110	4	13	9	9/9	147			
	108	5	6	5	5/5	183	swollen		
	104	6	14	12	12/10	155			
	101	7	9	7	7/5	171			
	98	8	10	10	10/0	0		white	sticky
8/68	156	0	23	20	20/20	526	pointed	dark	dry
15x19	152	1	11	11	11/5	25			
T.B.	148	2	31	28	16/12	247			
	145	3	27	26	16/15	344	round		
	142	4	31	31	16/15	383			sticky
	139	5	16	15	15/13	476	swollen		
	136	6	16	16	16/15	511		light	
	133	7	14	14	14/7	168			
	130	8	21	18	16/10	213			
9/68	186	0	14 –	4	4/4	39	pointed	dark	dry
17x19	183	1	13	7	7/7	66			
T.B.	178	2	16 –	8	8/8	119			
	175	3	28	20	20/18	315			
	172	4	22	18	18/18	401	round		
	169	5	20	20	20/20	545			
	166	6	13	9	9/9	98	swollen		sticky
	163	7	17 –	5	5/5	101		light	
	160	8	17 –	1	1/1	9		white	
10/68	279	0	9 –	3	3/2	8	pointed	dark	dry
19x15	275	1	22 –	2	2/2	3			
T.B.	271	2	17	15	15/15	415	round		
	268	3	11	11	11/11	442			
	264	4	16	14	14/14	610	swollen	light	sticky
	261	5	19	19	19/19	908			
	257	6	33	18	18/18	586			
	253	7	15 –	3	3/3	22			
	250	8	12 –	1	1/1	19			
12/68	467	0	9	8	8/8	111	pointed		dry
19x15	464	1	10	6	6/6	147			
S.O.	461	2	22	19	10/10	312	round		
	458	3	6	6	6/6	264			
	455	4	24	21	10/10	416	swollen		sticky
	452	5	16	13	12/12	542			
	449	6	37	22	10/10	291			
	446	7	18 –	9	8/7	96			
	444	8	16 –	3	3/3	36			
	440	9	4 –	1	1/1	37			

Appendix 1 (continued)

Test Cross Site[1]	Bag	Day[2]	Numbers of capsules: flowers pollinated	reaped	used/ with seed[3]	seeds	Stigma[4]		
13/68	500	0	18	10	10/7	30	pointed	dark	dry
10x6	497	1	17 –	6	6/5	61			
S.O.	494	2	17	12	12/11	126			
	491	3	18	16	16/16	489			
	488	4	19	14	14/14	120			
	485	5	17	14	14/14	460	round		
	482	6	20	13	13/13	541		light	
	479	7	10	6	6/6	290			
	476	8	14	14	14/14	611	swollen		sticky
	473	9	19 –	9	9/9	375			
	470	10	21	11	11/11	499		white	
14/68	530	0	20	19	19/11	35	pointed	dark	dry
15x6	527	1	12	11	11/6	21			
S.O.	524	2	17	11	11/4	11			
	521	3	22	20	20/19	367			
	518	4	31	30	20/19	727	round		
	515	5	22	21	20/18	824			
	512	6	33	29	19/18	1123			
	509	7	22	20	20/20	1162		light	sticky
	506	8	20	19	19/19	872	swollen		
	503	9	25	19	19/18	863		white	
	500	10	25	23	20/14	701			

[1]T.B. and S.O. refer to the tree bank and seed orchard sites respectively.

[2]The heading "Day" shows the number of days from emasculation of mature buds to pollination. If the series of days from 0 to 10 is incomplete, this indicates that no pollination was done on the days omitted from the series.
Lack of an entry under flowers pollinated shows that although pollination was done for that day, no capsules were reaped, the losses being attributed to mechanical damage. In those cases where the number of flowers is shown but with a nil entry for capsules reaped, no mechanical damage was observed.

[3]The number of capsules with seed was not observed in Test 1/66.

[4]The entries under the heading "Stigma" refer to the shape, colour and condition of the surface of the stigma.

In Tests 2/67 & 3/67, bags 44 and 45, the branches were broken and capsules were reaped at age 4,2 months.

Hyphens after the entry of numbers of flowers pollinated indicate heavy capsule losses of 50% or more, not attributed to mechanical damage.

Test 8/68, day 0, 6 of these 23 buds were pollinated an estimated 1 to 2 days before anthesis so that at least 3 of these were included in the 20 capsules which were reaped, and which had seed.

Appendix 2

Stigma receptivity on the basis of numbers of pollen grains, germinated and ungerminated, on stigmas pollinated during the first four days after anthesis

Days from anthesis to pollination	<1[1]	1	2	3	4
G10x17 Hand sections. Serial Nrs. (1969)	196	199	204	208	210
Nr. of stigmas examined	3	8	4	2	1
Nr. of stigmas with germinating pollen grains	0	1	0	2	1
Nr. of ungerminated p. grains	140	530	350	250	190
Nr. of germinated pollen . .	0	1	0	10	5
G15x(1+9) Stigmas "scraped" Serial Nrs. (1970)	263	259	255	251	247
Nr. of stigmas examined	3	3	3	3	3
Nr. of stigmas with germinating pollen grains	0	0	0	2	3
Nr. of ungerminated p. grains	1000	3200	1600	2100	2800
Nr. of germinated pollen . .	0	0	0	2	25
G17x(3+9) Stigmas "scraped" Serial Nrs. (1970)	268	264	260	256	252
Nr. of stigmas examined	3	3	3	3	3
Nr. of stigmas with germinating pollen grains	1	0	0	3	2
Nr. of ungerminated p. grains	1600	900	1600	3300	2000
Nr. of germinated pollen . .	1	0	0	8	79
G19x(3+9) Stigmas "scraped" Serial Nrs. (1970)	269	265	261	257	253
Nr. of stigmas examined	6	5	3	3	3
Nr. of stigmas with germinating pollen grains	0	0	3	3	3
Nr. of ungerminated p. grains	1400	2000	3200	3900	6300
Nr. of germinated pollen . .	0	0	156	356	430
G6x9 Stigmas "scraped" Serial Nrs. (1970)	270	266	262	258	254
Nr. of stigmas examined	2	3	4	3	2
Nr. of stigmas with germinating pollen grains	1	0	2	3	2
Nr. of ungerminated p. grains	700	1200	2400	3800	2900
Nr. of germinated pollen . .	1	0	49	315	161
Total					
Nr. of stigmas examined	17	22	17	14	12
Nr. of stigmas with germinating pollen grains	2	1	5	13	11
Nr. of ungerminated pollen grains (hundreds)	48	78	92	134	142
Nr. of germinated pollen grains	2	1	205	691	700

[1] <1 represents 12 hours after anthesis in G10, but the time of anthesis for the other clones.

The entries "hand sections" or "scraped" refer to the method of preparation of stigmas for examination (p. 72).

The serial numbers act as a guide to slide numbers.

Periods from pollination to sectioning, G15 10 hours
G6 12 hours
remainder 17 hours

Appendix 3

Number of pollen grains, germinated and ungerminated, on stigmas pollinated at anthesis and left for periods of up to five days before sectioning, compared with the same data from stigmas pollinated at maximum receptivity

Days from anthesis to pollination	0	0	0	0	0	4 to 5
G6x50 Hand sections						
Period from pollination to sectioning	1 days	2 days	3 days		5 days	6 to 9 hours
Nr. of stigmas examined	5	3	3		4	5
Nr. of stigmas with germinating pollen grains	0	0	0		4	5
Nr. of ungerminated grains	219	105	76		248	1080
Nr. of germinated grains	0	0	0		9	80
G17x15 Hand sections						
Period from pollination to sectioning	1 days	2 days	3 days	4 days	5 days	6 to 9 hours
Nr. of stigmas examined	3	3	4	2	2	1
Nr. of stigmas with germinating pollen grains	0	0	0	0	1	1
Nr. of ungerminated grains	730	193	421	210	114	1100
Nr. of germinated grains	0	0	0	0	2	32
G19x15 Hand sections						
P eriod from pollination to sectioning	1 days	2 days	3 days	4 days		6 to 9 hours
Nr. of stigmas examined	2	2	3	3		1
Nr. of stigmas with germinating pollen grains	0	0	0	3		1
Nr. of ungerminated grains	310	132	156	424		800
Nr. of germinated grains	0	0	0	15		54
Total						
Nr. of stigmas examined	10	8	10	5	6	7
Nr. of stigmas with germinating pollen grains	0	0	0	3	5	7
Nr. of ungerminated grains	1259	430	653	634	362	2980
Nr. of germinated grains	0	0	0	15	11	166
G6x50 Serial Nrs.	217	217	218		219	215
Slide Nrs.	1–5	6	3–5		1–4	1–3
		218				216
		1&2				1&2
G17x15 ..	237	237	237	238	238	236
..	1–3	4–6	7–10	1&2	4&5	
G19x15 ..	243	243	243	244		242
..	1–2	4&5	6–8	1–3		

Appendix 4

Numbers of flowers, capsules, seeds, seeds per capsule and relative yield of seed after self- pollination compared with cross-pollination

Test[1] (bag) Cross	Numbers of[2] flws. pol.	caps. reap.	% fruit set	Nr. of caps. used/with seed	Number of seeds[3] Ttl. x	f = per cap	Rel.[4] yield u	a	Nrs. sown/ survived in nursery
1/67 (31)									
6x6	11	7	64	7/1	6	0,9	3		
6x19	15	9	60	7/6	190	27,1			
6x0				7/7	111	15,9			
5/68 (24)									
6x6	40	21	53	21/16	138	6,6	30		138/73
6x19	24	9	38	9/6	198	22,0			198/69
6x0	53	36		18/17	129	7,2			129/50
12/69 (391)									
6x6	68	45	66	45/10	55	1,2	26		55/44
6xm	26	8		8/4	44	5,5			44/37
6x19	45	30	67	30/13	138	4,6			138/109
6x0	60	49		17/8	83	4,9			83/60
10x6	11	1		1/0					
10x19	17	14		3/3	19	6,3			
13/70 (701)									
6x6	20	11	55	11/0	0	0			194/168
6xM	32	7		7/0	0	0			59/55
6x19	27	14	52	14/2	6	0,4			97/76
6x0									269/222
18x6	21	13		13/13	738	56,8			
18x19	31	30		13/11	596	45,8			
Total G6 self	139	84	60	84/27	199	2,4	27		387/285
cross	111	62	56	60/27	532	8,9			433/254
open				42/32	323	7,7			481/332
3/67 (168)									
10x10	22	22	100	22/20	99x1,8=178	8,1		17	50/49
10x17	14	13	93	13/13	630	48,5			62/55
10x0				10/10	57	5,7			42/42
15x10	20	15		13/11	408	31,4			
15x17	12	12		10/10	569	56,9			
9/68 (350)									
10x10	58	38	66	38/29	57x0,6=34	0,9		1	55/46
10xm	22	17		17/17	649	38,2			57/52
10x15	20	20	100	17/17	1150	67,6			82/79
10x0	49	45		23/20	54	2,3			52/49
19x10	16	15		15/15	465	31,0			
19x15	23	22		20/20	388	19,4			

Appendix 4 (continued)

Test[1] (bag) Cross	Numbers of[2] flws. pol.	caps. reap.	% fruit set	Nr. of caps. used/with seed	Number of seeds[3] Ttl. x f = per cap.	Rel.[4] yield u a	Nrs. sown/ survived in nursery
17/70 (865)							
10x10	22	20	91	20/6	8x1,3=10 0,5	4	97/71
10xM	35	35		34/25	130 3,8		99/76
10x17	25	24	96	24/13	292 12,2		57/53
10x0							118/105
15x10	12	12		12/9	545 45,4		
15x17	11	10		10/10	576 57,6		
Total G10 self	102	80	78	80/55	222 2,8	7	202/166
cross	59	57	97	54/43	2072 38,4		201/187
open				33/30	111 3,4		212/196
8/68 (206)							
15x15	35	32	91	32/25	121 3,8	8	112/95
15x17	22	20	91	20/20	926 46,3		191/107
15x0				25/12	64 2,6		60/52
10/68 (376)							
15x15	44	30	68	30/20	69x1,6=110 3,7	7	
15xm	20	19		18/18	639 35,5		
15x10	18	14	78	14/14	778 55,6		
15x0	88	84		30/28	94 3,1		
19x15	23	22		20/20	388 19,4		
19x10	16	15		15/15	465 31,0		
15/70 (795)							
15x15	24	24	100	24/20	123x2,0=246 10,3	19	119/107
15xM	29	29		29/24	963 33,2		193/178
15x17	23	22	96	22/22	1166 53,0		101/91
15x0							193/169
6x15	21	15		15/10	276 18,4		
6x17	22	22		22/16	798 36,3		
Total G15 self	103	86	84	86/65	477 5,5	11	231/202
cross	63	56	89	56/56	2870 51,3		292/198
open				55/40	158 2,9		253/221
4/67 (184)							
17x17	22	5	23	5/2	2x2,2=4,4 0,9	3	
17x10	13	12	92	5/5	159 31,8		
17x0				6/5	11 1,8		
6x17	14	12		12/12	283 23,6		
6x10	7	7		7/7	356 50,9		
7/68 (55)							
17x17	61	16	26	16/4	5x1,3=6,5 0,4	2	4/4
17x19	33	28	85	28/27	572 20,4		23/18
17x0	48	14		3/2	42 14,0		42/34
6x17	11	8		8/8	255 31,9		
6x19	11	11		11/11	449 40,8		

Appendix 4 (continued)

Test[1] (bag) Cross		Numbers of[2] flws. pol.	caps. reap.	% fruit set	Nr. of caps. used/with seed	Number of seeds[3] Ttl. x	f = per cap.	Rel.[4] yield u	a	Nrs. sown/ survived in nursery
11/68 (550)										
17x17		56	23	41	23/9	18	0,8	2		
17xm		40	36		23/23	569	24,7			
17x6		27	18	67	18/18	752	41,8			
17x0		35	30		24/24	118	4,9			
16/70 (816)										
17x17		37	32	86	32/23	60x0,5 =30	0,9		2	57/47
17xM		39	28		28/27	635	22,7			65/61
17x15		40	22	55	22/22	830	37,7			91/85
17x0										135/119
6x17		22	22		22/16	798	36,3			
6x15		21	15		15/10	276	18,4			
Total G17	self	176	76	43	76/38	58,9	0,8		3	61/51
	cross	113	80	71	73/72	2313	31,7			114/103
	open				33/31	171	5,2			177/153
2/67 (68)										
19x19		6	6	100	6/3	4x0,09 =0,4	0,07		2	
19x6		11	11	100	11/9	39	3,5			
19x0					19/11	55	2,9			
17x19		15	8		8/8	181	22,6			
17x6		15	3		3/3	6	2,0			
6/68 (44)										
19x19		44	39	89	39/34	87x0,8 =70	1,8		4	83/63
19x17		34	30	88	30/30	1370	45,7			185/100
19x0		23	20		10/9	84	8,4			81/64
6x19		11	11		11/11	449	40,8			
6x17		11	8		8/8	255	31,9			
14/70 (731)										
19x19		36	21	58	18/11	15x1,2 =18	1,0		3	19/11
19xM		31	19		19/19	170	8,9			69/60
19x6		19	19	100	19/19	622	32,7			35/32
19x0										34/20
18x19		31	30		13/11	596	45,8			
18x6		21	13		13/13	738	56,8			
Total G19	self	86	66	77	63/48	88,4	1,4		4	102/74
	cross	64	60	94	60/58	2031	33,9			220/132
	open				29/20	139	4,8			115/84
18/71 (100)										
58x58		146	48	33	47/35	105				105/55
58xM		43	29		6/6	185				185/151
58x19		46	28	61	11/11	232				232/206
58x0		87	65		17/17	197				197/182

Appendix 4 (continued)

Test[1] (bag) Cross	Numbers of[2] flws. pol.	caps. reap.	% fruit set	Nr. of caps. used/with seed	Number of seeds[3] Ttl. x	f = per cap.	Rel.[4] yield u	a	Nrs. sown/ survived in nursery
19/71 (262)									
1x1	156	136	87	136/					/802
1x5X	37	28	76	28/					/512
1x0									/630
20/72 (317)									
1x1	46	24	52	24/24	209	8,7	30		208/74
1xM	31	15		15/14	300	20,0			300/159
1x5X	15	8	53	8/8	235	29,4			235/69
1x0				50/	221	4,4			221/131
21/72 (342)									
30x30	35	19	54	17/17	171	10,1	32		167/79
30xM	18	15		15/15	350	23,3			348/196
30x5X	12	10	83	10/10	311	31,1			299/159
30x0	24	16		16/16	137	8,6			130/96
15x30	7	3		3/1	4	1,3			
15x5X	4	4		4/4	108	27			
22/72 (373)									
15x15	8	8	100	8/7	40	5			40/29
15xM	7	6		6/6	175	29,2			175/150
15x5X	4	0							
15x0	30	26		26/23	134	5,2			134/126
23/72 (415)									
24x24	54	16	30	16/12	28	1,8	7		28/23
24xM	32	17		8/8	155	19,4			45/34
24x5X	42	30	71	6/6	145	24,2			92/73
24x0				2/2	51	25,5			51/43
24/72 (393)									
13x13	63	42	67	42/42	440x2,5=1100	26,2		47	236/208
13xM	33	21		21/21	597	28,4			217/193
13x5X	21	11	52	11/11	616	56,0			115/107
13x0				21/21	171	8,1			171/159
45x13	9	7		7/7	61	8,7			
45x5X	11	11		7/7	151	21,6			
25/72 (301)									
34x34	61	18	30	18/18	179	9,9	35		179/162
34x5X	65	30	46	6/6	171	28,5			103/83
34x0				12/12	190	15,8			190/174
26/72 (360)									
9x9	30	17	57	17/17	66	3,9	19		66/62
9x5X	17	6	35	6/6	123	20,5			55/53
9x0				8/8	41	5,1			41/40
27/72 (438)									
1x1	26	23	88	23/23	208				208/196
1x0	36	27	75	27/27	233				233/195

footnotes/.....

Appendix 4 (continued)

[1]The column headed Test (bag) shows the test number/year of pollination and under that, in parentheses and as a cross reference to the pollination record, the first pollination bag number of each test. The crosses are shown under these headings and in each test, the first clone listed as female represents the main test and, where present, the second clone listed as female represents the pollen test (p. 84):m, mixed-pollination using the self and cross pollens mixed in approximately equal proportions; M, mixed-pollination as above but mixing done in equal proportions by weight; 5X, cross-pollination using pollen from five different clones mixed in approximately equal proportions and O, open pollination.
Cross pollens were, in Test 19, G17, 19, 38, 58 and 101
. . . . . , in Tests 20 to 26, G16, 17, 19, 47 and 101.

[2]The numbers of flowers shown as pollinated ("flws. pol") exclude losses seen to be due to mechanical damage (p. 22).

[3]The heading "number of seeds" refers to the numbers germinated. Under the sub-heading "Ttl. x f =" are shown the actual number of germinations, a factor derived from the pollen test and, in the case of the selfs, the adjusted numbers resulting from application of the factor to the actual number of germinations.

[4]Relative yields are shown as "u" unadjusted, or "a" adjusted, depending on whether results of a pollen test were applied.

Seeds were transferred from the pads of the germinator to planting tubes as soon as they had germinated except in Tests 3 to 11, in which they were left on the pads for observation for a day or two (p. 84).

In Tests 3, 6 to 9 and 23 to 26 in which random discard of excess seed was done (p. 85) the numbers recorded as sown (under the right hand column) are the numbers of germinating seeds which were transferred from the pads of the germinator to planting tubes. In other tests, where no discard was done, except as stated in the next paragraph, the numbers shown are those which germinated on the pads.

In Tests 20 and 21, some sowings were discounted to allow for unusually heavy insect and other damage (p. 25).

In Tests 13 to 17, the numbers sown/survived are from Tests 13B to 17B, but all other data are from the concurrent Tests 13A to 17A (p. 83).

In Tests 10 and 11, survival figures are not entered because of some deaths caused by unauthorised application of fertiliser, and in Tests 1, 2 and 4 because numbers were too few to merit planting.

In Test 19, no germination record was kept, only a record of the numbers of seedlings which survived in the planting tubes long enough to be classified as normal or deviant.

In Tests 18, and 27 the pollination was not designed for relative yield purposes.

Tests 6 and 7, 9 and 10, 13 and 14, 15 and 16 were done in reciprocal pairs (p. 84).

Appendix 5

Incompatibility on the stigma from counts of germinations of pollen on receptive stigmas after self-pollination compared with cross-pollination

Test 1 Bag 1000

	Main test				Pollen test	
Cross	15x15	15x19	19x19	19x15	6x15	6x19
Serial numbers	272	271	274	273	275	276
Numbers of stigmas examined	5	4	5	4	6	5
ditto with germinating pollen grains	5	3	5	4	6	5
Numbers of ungerminated grains (hundreds)	29	15	23	31	42	9
Numbers of germinated grains	16	5	22	68	99	52
Germinations per stigma	3,2	1,3	4,4	17	17	10
Factor[1]						1,7

Test 2 Bag 1021

Cross	10x10	10x19	19x19	19x10	17x10	17x19
Serial numbers	279	280	277	278	281	282
Numbers of stigmas examined	5	5	6	5	5	5
ditto with germinating pollen grains	5	5	6	5	5	5
Numbers of ungerminated grains (hundreds)	119	78	86	117	100	49
Numbers of germinated grains	596	257	51	281	346	360
Germinations per stigma	119,2	51,4	8,5	56,2	69	72
Factor[1]						1,04

Test 3 Bag 71

Cross	10x10	10x15	15x15	15x10	6x10	6x15
Serial numbers	283	284	285	286	287	288
Numbers of stigmas examined	5	6	6	6	4	4
ditto with germinating pollen grains	5	6	6	6	4	4
Numbers of ungerminated grains (hundreds)	74	61	129	117	95	23
Numbers of germinated grains	961	524	936	1462	1347	119
Germinations per stigma	192,2	87,3	156	243,7	337	30
Factor[1]						11,2

[1] Factor as used in Table 27 (p. 109) in applying results of pollen tests.

Period from pollination to sectioning, in Tests 1 & 2, 10 hours
in Test 3 15 hours.

Stigmas prepared by scraping (p. 72) in Tests 1 & 2, no record for Test 3.