SILVICULTURAL RESEARCH - BOTANICAL.

Report for 1950. by J.H. Harding

It is unfortunate that precedents are not available in the field of Forestry in anything approaching the abundance and variety met with in Agriculture and Horticulture. This is understandable in view of the difference in life cycle and the many other difficulties associated with large scale tree culture.

In recent years fundamental work has been accelerated and useful contributions are being made in the fields of breeding, quality, association, general silviculture, soils and site generally, physical and chemical properties, etc.

The importance of forest products in the economics of living is the leading influence, coupled as it is with the growing world wide consciousness of the rapid depletion of world supplies. This realisation has been delayed in the past by the fresh forest resources in the New Worlds producing a new abundance, but the time has now arrived when there are no extensive new areas available. Each country must now view its forest resources on the same plane as agriculture and adjust the balance accordingly.

This involves extensive research and improvement in all phases of silviculture.

Much of the work involved in this type of research is exploratory and of a long term nature. However, there are certain aspects which can be dealt with in fairly short order.

Work done to date can be summarised as follows:-

1. Pinus species.

   (a) Anatomical
   (b) Nutritional
   (c) Cytological
   (d) Vegetative
   (e) Pathological
   (f) General

2. Eucalyptus species.

   (a) Differentiation of Jarrah and Karri
   (b) Pathological
   (c) Site and Physiological
   (d) General

3. Miscellaneous.

Details of work follow.

Section 1. PINUS SPECIES.

(a) Anatomical.

Lord Robinson, Chairman of the British Forestry Commission, referred samples of P. radiata "dead top" material taken when in W.A. to his wood anatomy branch for investigation.

The report received stated that the appearance of the wood was similar to frost-damaged material. This was, of course, not the case, but was a rather interesting viewpoint.

Symptoms were an apparent increase in resin ducts, black ring surrounding the wood just inside the bark, fairly numerous cracks in radial direction with collapse present in outer cells.

The assumption is that this damage is caused by heat, not cold. This is subject to verification. The black ring appeared to be an oxidation product of resin and sap.

No pathogenic primary appeared to be present either the dead wood or in the unthrifty dying tops of sick trees.

It is of interest to note that responses have not been obtained on laterite either with zinc or phosphate, and it was thought that hormone treatment might prove effective.

(b) Nutritional.

Zinc spraying was done on a patch of sick P.radiata at Gleneagle on laterite on which solids were applied as detailed below at the same time. This work was done to parallel hormone injections in adjacent trees. Results are not yet available due to the short period since treatment, but there is a suspicion of better colour in the sprayed series.

Hormone injection treatment.

Six trees between 5.5" and 17.25" G.B.H. all very sick, yellow needled, rosetted and sparse crowned treated with varying quantities of special hormone solution.

The solution was specially prepared by Warner's laboratory and contained 10 hormone compounds and 23 trace elements as a blanket treatment. Contents not known, but arrangements have been made for disclosure of composition if a response is obtained.

Solids.

(a) On 1 sq. chain plot - combined fertiliser:-

11.2	lbs.	Super
11.2	lbs.	K_2SO_4
11.2	lbs.	$MgSO_4$
1	lb.	$FeSO_4$
2	lbs.	$ZnSO_4$
1	lb.	$MnSO_4$
1	lb.	$CaSO_4$
½	lb.	Bo

(b) Heavy applications of $ZnSO_4$ and $CaSO_4$ six buffered trees in each series. Trees received 5 lbs. of salt over 3 foot radius from butt.

Spraying.

Foliage of each tree wetted with 2½% aqueous solution of $ZnSO_4$ from a packspray.

These experiments are all exploratory and detailed descriptions, G.B.Hs and heights have been taken. Remeasurement will be done in 12 months and compared with previous M.A.I.

Visual inspection for improvement in colour and foliage density will be made periodically.

(c) Cytological.

This subject was approached first on 11th August, 1948, when 100 1/0 P. pinaster (Leiria strain) seedlings were lifted and 50 of these were treated with 0.2% Colchicine in an attempt to induce polyploids.

The technique employed was to soak small pledgets of cotton wool in the colchicine and apply to the leading shoot, renewing every 3 hours. Treatments were carried out to exhaustion of 50 c.c. of solution and 10 applications were made.

The remaining 50 seedlings were given a similar treatment using local tank water.

All seedlings were bagged in the normal manner and kept in a moist, shady spot during treatment. Planting was done on 13.8.48 alternating treatments ~~simply~~ SINGLY to give even environmental influence to each treatment. "Colchicine" trees were later marked with white pegs and plants were watered during dry periods in the ensuing summer. Takes were about 96%.

Periodical inspections did not give any concrete indications until 24.10.49 when 4 trees showed tendencies towards polyploidy. These were -

1. Vigorous and massive leader growth.
2. Deep pigmentation of new shoots.
3. Depressed lateral growth.
4. Rosetting at leader terminals.

Growth was not sufficient for shoot material to be taken so it was decided to wait until 1951 before attempting to make cytological tests. (See Appendix II).

The second approach was made in January 1950 when shoot material was tested for division from samples taken at 11 a.m. and 3 p.m. Aceto Loemoid and iron aceto-carmine stains were used following fixation with acetic alcohol. This study showed no division occurring at these times.

The third approach, which was really a continuation of the second series, was made at Somerville on 16.1.1950, when shoot material was collected at 2-hour intervals commencing at 7 a.m. and finishing at 7 p.m. Squashes of this material from formalin acetic alcohol showed no sign of division, but early telephase was seen in some cells.

The fourth approach was made at Somerville on 20.1.1950 when shoot material was collected at hourly intervals from midnight until 7 a.m. the next day.

Squashes made from this material out of formalin acetic alcohol using iron aceto carmine stain showed prophase commencing after 1 a.m. and anaphase to telephase at 0600. The chromosomes were too long and overlapped for satisfactory counts to be made and it was decided to defer counts to the next flowering period. (See Appendix I covering the full procedure on this fifth approach).

The data obtained in the four approaches listed above established that mitotic division occurred between 1 a.m. and 7 a.m. in January, and it was considered this was probably influenced by water relations being better at this period. No proof was obtained on this aspect.

Further, it was established that the stains used were quite suitable, but that the small cells and long chromosomes made it difficult to observe numbers. This led to a deferment of proposed mitotic work on the suspected polyploid series of Approach 1. until the next flowering period.

(d) Vegetative Work.

Jacobs (C.F.B. Bull. 25, 1939) has successfully raised P. radiata from terminal cuttings prior to formation of male catkins, but results after this stage have been negative. The reason for this is a subject for investigation. The implication is, of course, that unless pedigree stock is available, the desirability of parent trees cannot be fully judged at this darly stage. Further work is proceeding at Canberra.

Similar work is being done in various other countries, notably California and Denmark.

P. pinaster has not been a satisfactory subject in this regard although it is understood that a successful technique has been developed in Europe. Details of this are not to hand.

Some work has been done in this State commencing in 1948 aimed at testing root stock possibilities of P. pinaster with P. radiata scions.

Six successful approach grafts of 2/o seedlings of these species were made at Ludlow in January 1949, and these plants were set out in the next planting season. Unfortunately they did not survive the very dry summer of 1950.

A further six grafts of 2/o P. radiata seedling leaders were done on 4-year Leiria stock in the same period, while in late January a series of grafts in P. radiata were made using scions from female fertile shoots of old trees on 4-year old trees of the same species.

The former grafts were successful in 4 cases where pruning of the P. pinaster leader was left for 8 weeks or more.

In two cases pruning of the P. pinaster leader was done at 4 weeks and the scions died.

In the case of the P. radiata grafts, the idea was to see whether cone material could be produced at low altitude. Unfortunately all except one of these grafts received stock damage. The remaining case was growing vigorously in August 1950, but had not developed flowering material.

In the case of P. radiata on P. pinaster in August 1950, two grafts remained since a further death and damage possibly due to spraying one other with zinc caused another death.

In the case of the two survivals and the last-mentioned death, the scions were showing typical zinc deficiency symptoms, although the P. pinaster stock was quite healthy. This is most interesting and zinc will be further tried when the two remaining scions are more advanced next year. A further interesting feature is

that both these scions have borne male catkins this year, although they are very young and were originally leading shoots.

On 13.4.50 seven elite P. radiata trees central paddock Stirling were selected and climbed, mature cones and shoot material being collected. Shoot material was grafted on two year old P. radiata stock in Compt. 33 Horse Paddock, Stirling. Unfortunately stock damage and breakdown of rubber bands used in lieu of raffia on U.S. specifications caused complete loss of the 23 grafts involved.

Seed was extracted from cones collected and carefully bagged and recorded with parent tree number. This seed was germinated and potted and will be used as a basis of a progeny trial.

(e) Pathological.

This was divided into two series, the first being disorders of established trees, and the second, nursery disorders.

With mature trees, a case of death of a reasonably extensive patch of trees was observed at Greystones 10. and it was thought that Sirex wasp or some fungal attack might be the causative. No evidence was forthcoming to support either theory and the cause is thought to be some physiological aspect which is listed for further investigation. A similar series of deaths occurred at King's Park and inspection of these trees revealed no pathogenic evidence. In this case it is assumed that proximity of limestone may be the cause. This trouble also is being further investigated.

At Somerville on Terra Rossa type soil an intense yellowing and numerous deaths occurred in seven year old P. pinaster (Leiria) which had previously shown no acute symptoms of disorder. The acute trouble was confined to upper slopes and ridges and again the proximity of lime stone is thought to be a contributing factor. A detailed soil study is in progress.

On the nursery side, some trouble has been experienced with damping off and root rotting. Trials are in progress using Copper Carbonate as a fungicidal dust on seeds in addition to and separate from the usual bird and rodent deterrant, Lead Oxide.

A series of pH determinations made at Gnangara showed the healthiest section of the nursery to be pH 5.3 while the unhealthy sections were in the range pH 5.9 to 7.0. Further work will be done by adjusting pH with sulphur applications.

A series of experiments is under way at Gnangara, Somerville and Ludlow using various fungicides to test their effect on the root rotting aspect, presumed to be a rhizoctonia caused trouble. Results will not be assessable until early next year. Fungicides under test are:-

Fermate
Mercury E
T.M.T.D.
Shirlan W.S.
Wettable Spergan

(f) General.

In this section is included seed testing and stimulation of germination and other investigations of a miscellaneous nature.

1. Chemical Determination of Viability.

Triphenyltetrazolium bromide (Grodex). (Annals of Applied Biology, Vol.35, No. 1, March, 1948) was used to test viability of three batches of Leiria seed, with the following results :-

Serial No.	Abortive	Staining				No. of Seed examined
		Red	Pink	Light Pink	Uncoloured or only Tinged	
685	19	78	116	87	21	321
726	37	168	75	45	16	341
750	15	260	56	15	4	350

Red and Pink could be regarded as viable.
Light Pink could be regarded as weakly viable.
The remainder are non-viable.

The percentages based on this test were :-

Serial No.	Viable	Weak and Non-Viable	Aborted
685	60.6	33.6	5.8
726	71.3	17.9	10.8
750	90.3	5.4	4.3

Germination trials with this seed are included as Appendix III, and observations will be noted in that section of the report.

2. Stimulation of Germination - detailed tables in Appendix III.

Leiria strain P. pinaster has shown a marked tendency towards semi-dormancy, that is, the spread of germination has been considerable, covering as much as 18 weeks or more. This is unsatisfactory in W.A. conditions of a short winter wet season usually confined to 4 months, and it is important that the main germination period should be confined to a period of no more than 8 weeks following spring sowing in late August.

3. Nursery Sterilisation.

(1) D.D. was tried as a steriliser and weed deterrent at Somerville, Ludlow and Gnangara, 12 x 12 exploratory plots were used. The application was 3.5 c.c. at 1 foot intervals at 6" depth.

The results assessed this year were as follows :-

(a) Sucker sorrel was eliminated but seedling sorrel persisted. This is important as burning off with a Hauck torch disposes of the seedling growth, but sucker growth is hard to control.

(b) There was some depression in annual weed growth but not sufficient to be regarded as effective.

(c) Pine seed sown under two weeks after treatment was badly affected, but satisfactory germination occurred if sowing was done after that period.

(d) It was intended that this steriliser should be tried on Couch infected areas, but unfortunately this was not done.

The general conclusions are that, with the exception of depression of sucker sorrel, no real advantage is obtained from this treatment and there is danger of loss of plants if the area is not completely free of D.D. residues prior to sowing.

(2) Zinc Sulphate as a weedicide. Results using 5% $ZnSO_4$ in South Australia indicated that a depression of sorrel growth was caused by this salt.

Strips were sprayed at Somerville and Gnangara, but results were obtained only on seedling growth, not sucker growth.

This treatment is fairly expensive and results are of no real importance in view of points raised in the previous section.

(3) Stratification. Experiments in 1949 indicated that germination could be stimulated in tardy germinating Leiria seed by subjecting the seed to 3 weeks' stratification at 1° - 2°C. in moist conditions (12% moisture added on a dry weight basis); the results were: Control 5%, Stratified 20.5% in 8 weeks. South Australia obtained similar results.

This year all spring-sown P. pinaster seed was treated in this way and control tests are listed in Appendix III.

Section 2. EUCALYPTUS SPECIES.

A.E. Differentiation of Jarrah and Karri.

(a) Tannin using Ferric chloride.

(b) Alkalinity of ash.

(c) Furfural (Penteran) Content.

(a) Tannin.

The method used was that outlined in C.S.I.R. Pamphlet No. 53, 1935, (W.E. Cohan) (Division of Forest Products Tech. Paper No. 15). Results using Jarrah from pure and mixed stands and Karri from various sections of a good Karri area are listed below.

Forest Type & Sample No.		Reaction Dilute		Reaction Concentrated		Extract Color
A. Pure Jarrah	1	Olive brown green	P	Blue	P.	Light brown
	2	Blue	P	Blue	P.	Light amber
	3	Blue	P	Blue	P.	Dark red amber
B. Mixed Jarrah	4	Blue	P	Blue	P.	Lighter than 3
Jarrah	5	Olive green	S.P.	Olive green	N.P.	Pale light brown
Karri Blackbutt	6	Blue	P	Blue	S.P.	As for 3.
C. Karri	7	Olive green	P	Olive green	P.	Very light brown
	8	Blue green	P	Blue	P.	Light brown
	9	Olive green	P	Bluish green	P.	Light amber

A. = Palgarup. B = Wilgarrup River E.S.E. of Manjimup Karri fringe.
C = Donnelly River, W. of Manjimup.

P = Precipitate
S.P. = Slight precipitate
N.P. = No precipitate of any consequence

This shows that the test is inconclusive and unreliable.

(b) Alkalinity of Ash.

Method outlined in same pamphlet as (a).

The following results were obtained using the same samples:-

Sample		Alkalinity ~~N/10~~ M/5 H_2SO_4 per gm. dry wood.	Average Alkalinity
Pure Jarrah	1	0.022	
	2	0.022	
	3	0.039	0.028
Mixed Jarrah	4	0.132	
	5	0.003	
	6	0.080	4 & 6 only 0.11
Karri	7	0.51	
	8	0.45	
	9	0.48	0.48

Values given by Pamphlet were :-

Species	Average	Maximum	Minimum
Jarrah	0.06	0.12	0.02
Karri	0.83	1.85	0.42

This test appears reasonably conclusive.

(c) Furfural Test.

Method outlined by W.E. Cohen and done by him on a different series of samples as his samples of those used by me for (a) and (b) had been used up in his check determinations.

Species & Forest Type	Furfural %	Mean
Jarrah close to Karri	5.71	
" pure	6.10	
" pure	6.25	
" Mixed with Marri & Karri	5.77	
" Pure	5.60	
" Mixed with Marri & Karri	5.12	5.76
Karri near rung area	10.18	
Karri pure	10.59	
Karri mixed with Marri	10.80	10.52
Blackbutt mixed with Marri	7.15	
" " " "	8.03	
" " " "	6.98	7.39

Means of species were significantly different, but means of samples within species were also different.

As a broad test this determination also appears conclusive.

Note: (1) The old bushman's test of burning a sliver of the timber is often satisfactory but may lead to error in view of the above results.

(2) The equipment for the last test was not available and Mr. Cohen undertook the work.

B 2. Pathological.

Dark stains were noted in the inner sapwood areas of "die back" jarrah at Gleneagle in May, 1950. Investigation by the Department of Agriculture Plant Pathologist and Plant Entomologist was negative.

Sections cut by me showed no pathogenic indications but the ray tissue was packed with oval shaped smooth starch grains. This was rather interesting to note in a tree very close to death.

Further work is in progress.

C 3. Site and Physiological Aspects in the Jarrah Forest Die-back Areas

(a). pH determinations of 30 soils samples at Teesdale were done, but the significance of these is not yet realised. The work being part of a project being carried out at Dwellingup Research Station.

(b). General directions were set out by me for an investigation into the psychological and anatomical effects of Fire Damage on Jarrah regrowth at Dwellingup. The work is being done to date by Dwellingup.

D 4. General.

As a part of the restoration of Crown Studies it was decided to try effects of Hormone injections and a similar solution to that outlined in the Pinus series (3a.).

Six replications of three treatments with a unit of 10 trees were used, the area being one mile south of Gleneagle H.S.

The design was randomised blocks and the site was a sheer laterite ridge carrying good jarrah with sparse poor crowns. A few marri were interspersed in the area. The understory was ~~mixed~~ Banksia with occasional Persoonia. The ground flora was mixed typical lateritic types including Myrtaceae, Proteaceae, Legumes and Rutaceae. Sedges were occasional and numerous herbs and annuals occurred. The forest litter was good (about 8 years' accumulation).

Treatments 1. 10 ccs. of Hormone solution
2. 10 ccs. of distilled water.
3. Untreated.

Details taken: G.B.H.
Height
Log class
Canopy class
Crown class
General description of any other features of the tree.

Reassessment: 1. G.B.H. at 4-weekly intervals
2. General observation of appearance at similar periods.

Section 3. - GENERAL WORK.

During the 12 months just passed, the following work has been done :-

1. A course of 3-hours lectures on general botany has been given to (a) District Foresters' School
(b) Foresters' School

2. Microtome work on woody stems has been done.

3. Transplanting of indigenous shrubs has been carried out using the tin cylinder method to enclose soil and retain a fair section of the root system.

The points observed were:-

(a) Seedlings were selected when foliage could be definitely identified from adjacent mature plants.

(b) Care was taken that the main root system was not damaged by the cylinders.

(c) Plants when lifted were immediately placed under cover and kept watered during transit.

(d) On arrival at the planting site, plants were kept moist and under cover for a week prior to planting out.

(e) Watering of plants as necessary has been arranged for.

Note. 1. The orientation of plants has been advocated as important, but this was not given attention.

2. If these transplants are successful, they will form the precursors of a 10-acre arboretum of local species at Gnangara.

4. Ecological Studies.

(a) Grazing quadrants at Kings Park have been assessed and plotted, see bound volume attached.

(b) Further work will be done next month to see the effect of grazing during September and October.

(c) Arrangements and details of gridding and assesement criteria were made for a census of Veldt grass distribution in Kings Park.

5. Identification of Wood Samples.

Various identifications have been made. In this regard Forests Products Research Bulletin No. 22 "Identification of Softwoods", E.W.J. Phillips, 1948, Dept. of Scientific & Industrial Research, Princes Risborough, has proved very useful.

Perth
17th November, 1950.

APPENDIX I

PINUS PINASTER IN WESTERN AUSTRALIA.

This report is concerned primarily with the fundamental cytology of the species as represented in this State, but the question of strain purity has received some consideration on account of its practical importance.

Perry (1), (2), has explained that six so-called strains can be recognised and of these five are under observation in this State. His classification is based on origin of seed and the rather full notes given by Duff (3) have influenced him to a considerable degree in clarifying his concepts of the species and its strains.

The question of nomenclature might be briefly touched upon here as it has been suggested that well defined types might be worthy of specific names. It does not, however, appear so to the writer, rather would it seem that the species name pinaster followed by a variety name would meet the cases concerned. This aspect is not of great importance to the present study at this stage beyond its possible implication of fundamental cytological differences being present.

Perry's classification is as follows:-

Atlantic Race.

(a) Landes Strain
(b) Portugese or Leiria Strain
(c) French Hoek (South Africa)

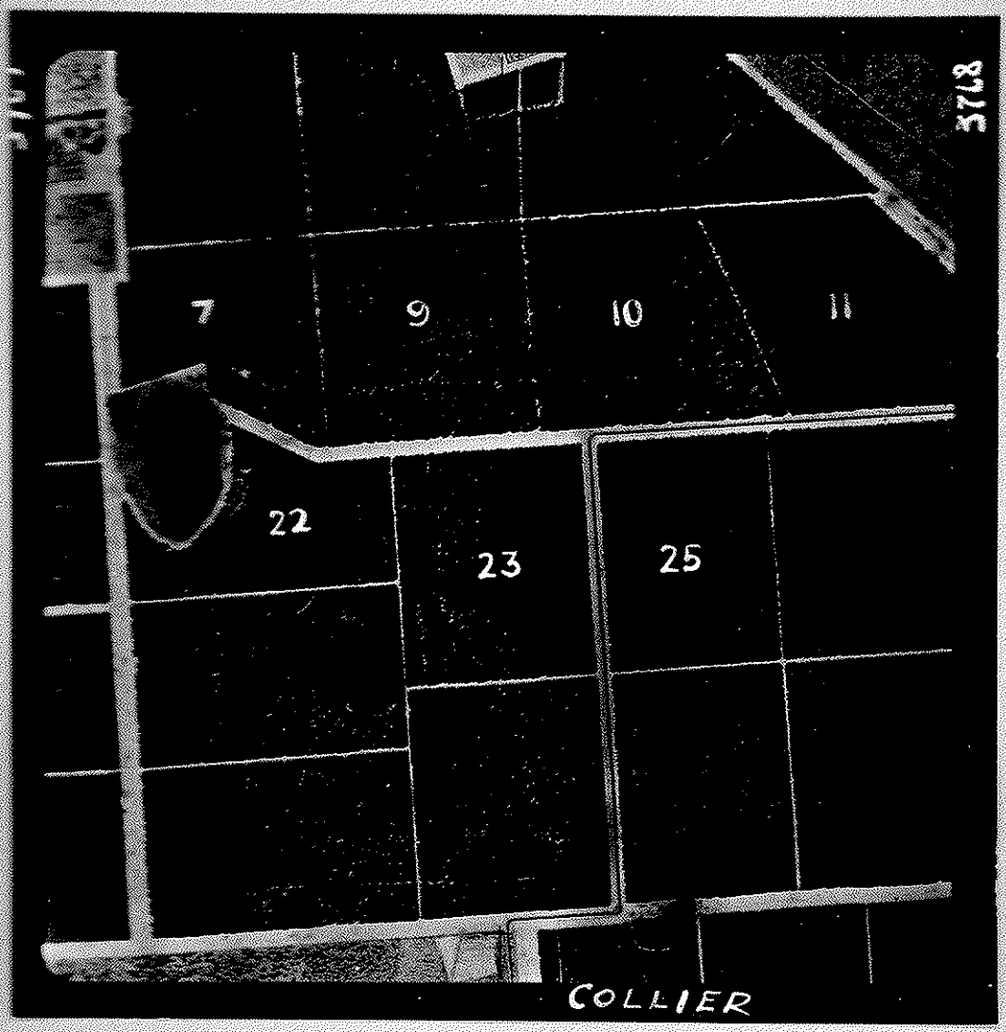
Mediterranean Race.

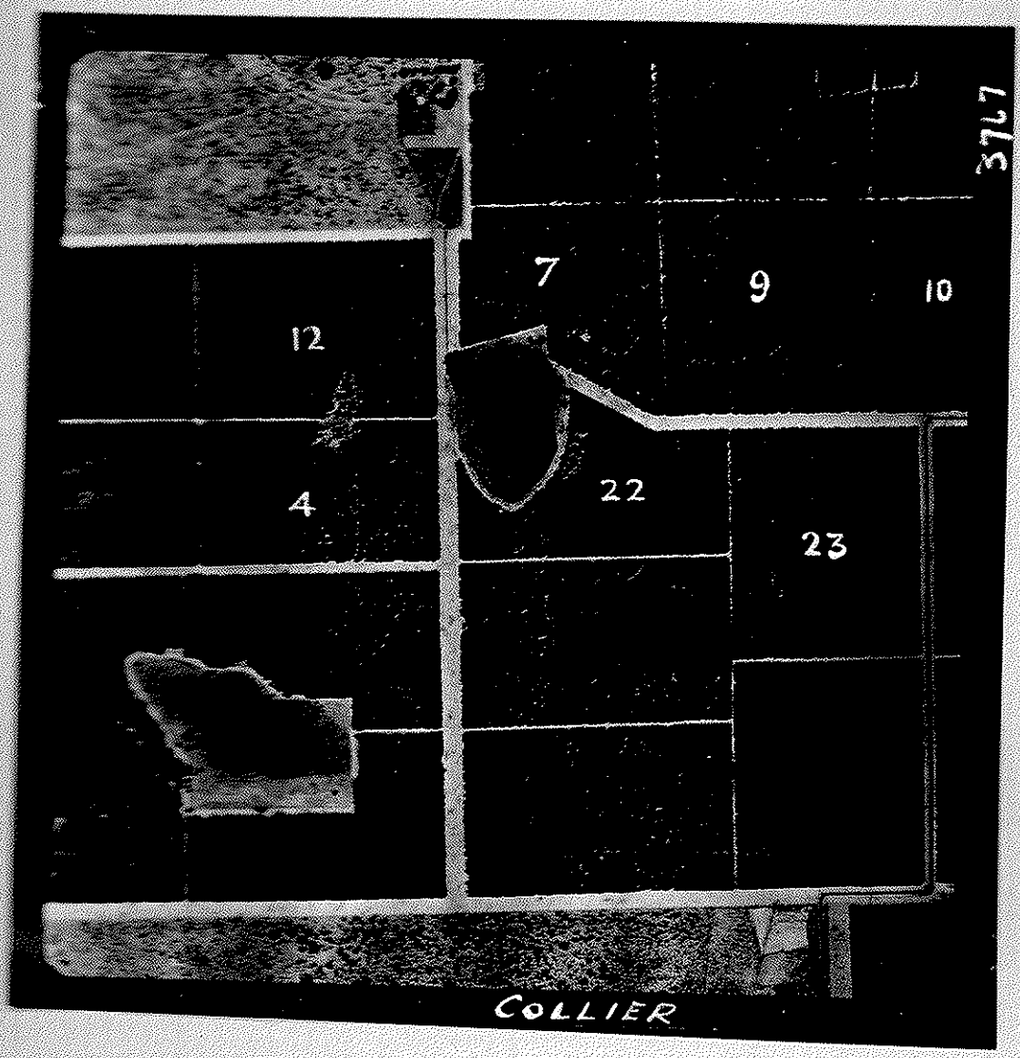
(a) Esterel Strain
(b) Corsican Strain
(c) Lucca or Italian Strain

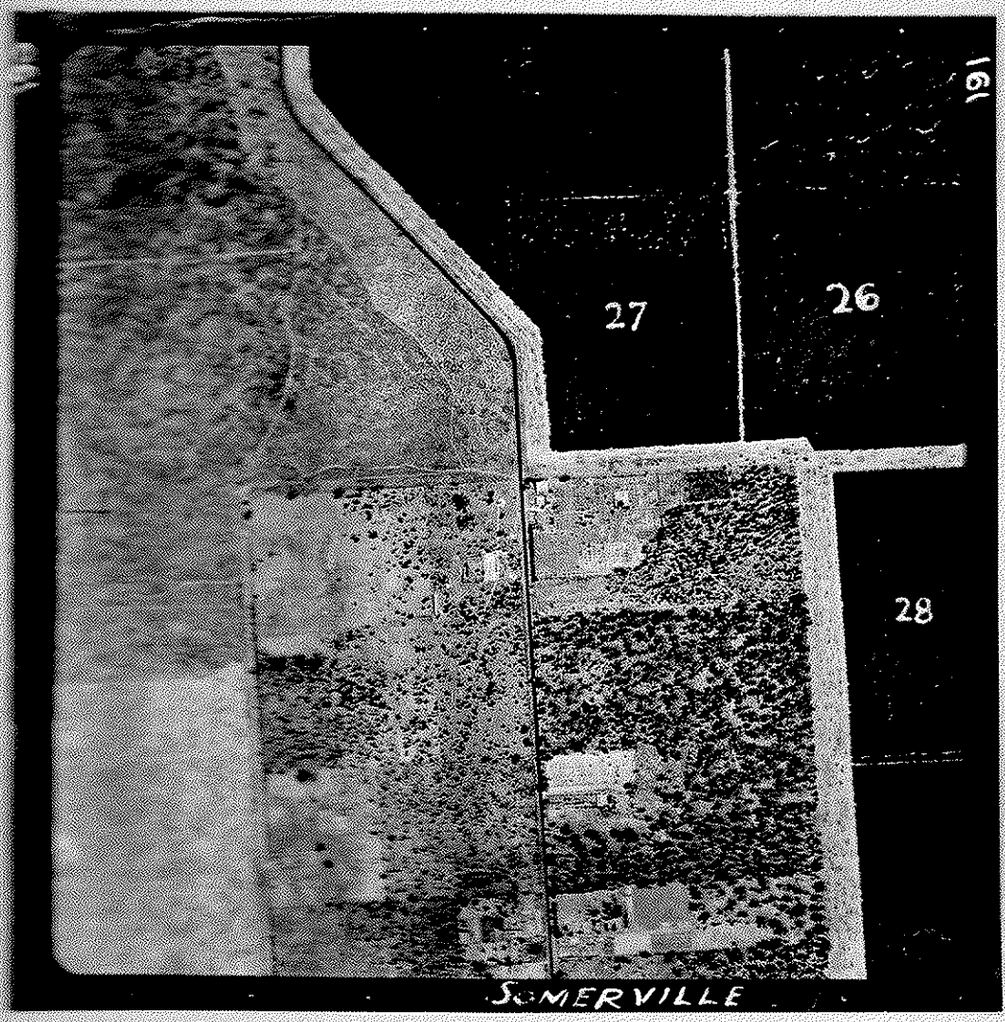
Strains (a) and (c) in the Atlantic Race show a wide range of form and many fine trees are included in the former type.

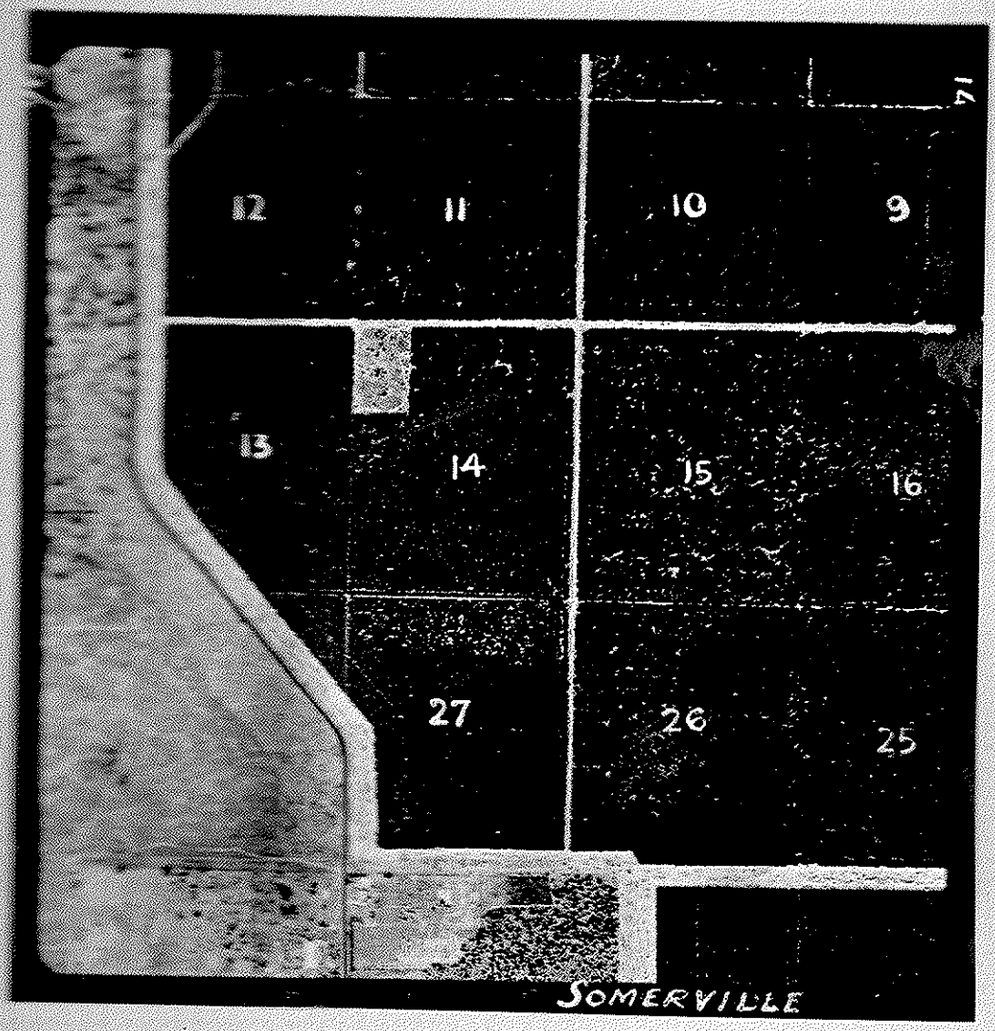
Photographs of trees typical of the general form in each strain as it is known here follow.

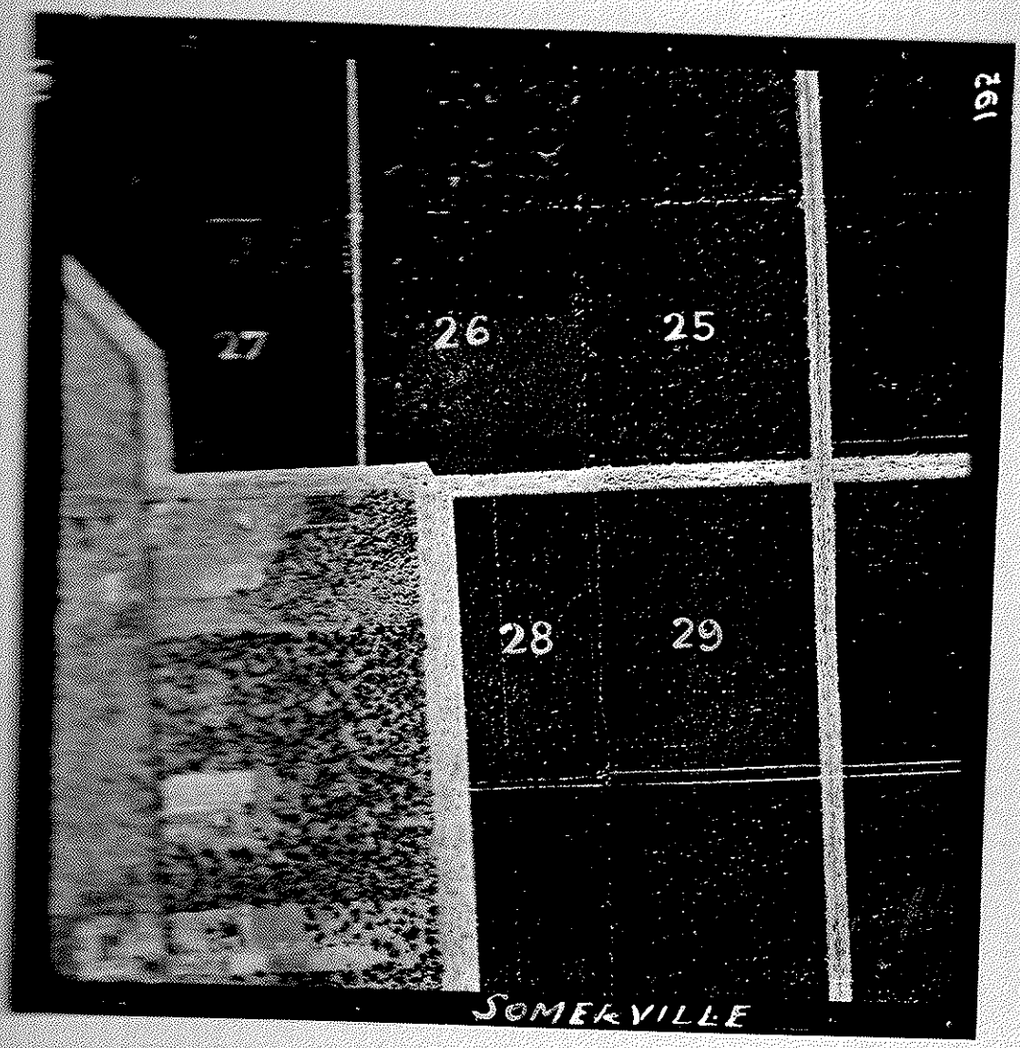

20
21
19
16
3
4
2
GNANGARA

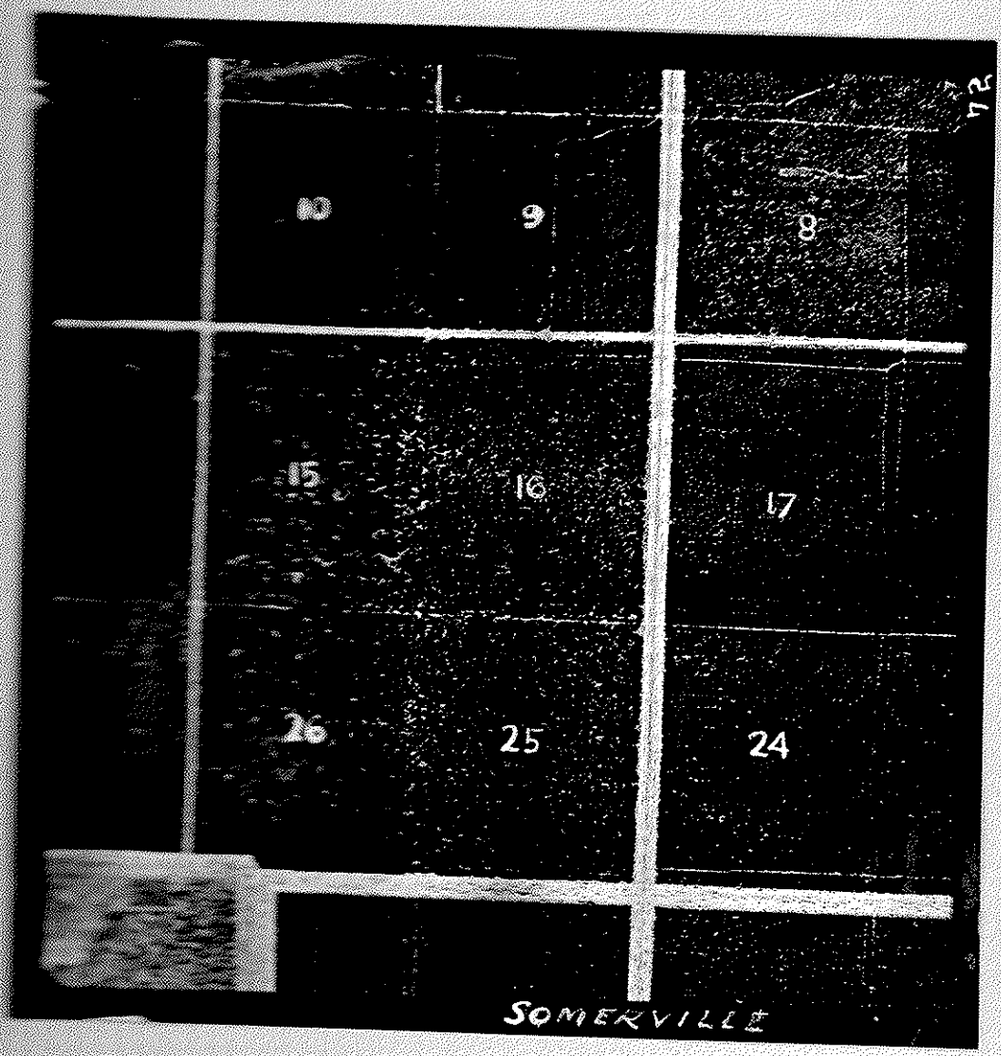

3718
7
9
10
11
22
23
25
COLLIER

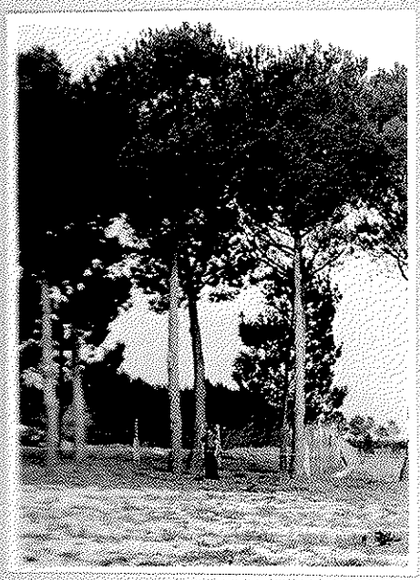

3747
7
9
10
12
4
22
23
COLLIER


191
27
26
28
SOMERVILLE


12
11
10
9
13
14
15
16
27
26
25
SOMERVILLE


192
27
26
25
28
29
SOMERVILLE


10
9
8
15
16
17
26
25
24
SOMERVILLE



Gnangara Headquarters.

P.pinaster, planted 1922.
Mediterranean.

Gnangara Headquarters.

Similar to 1

Ludlow C.28.

Mixed esterel and lucca; Mediteranean. Broadcast sown April 1920. Planted July 1921; re broadcast May 1924.

As for previous photo.

Ludlow C.28.

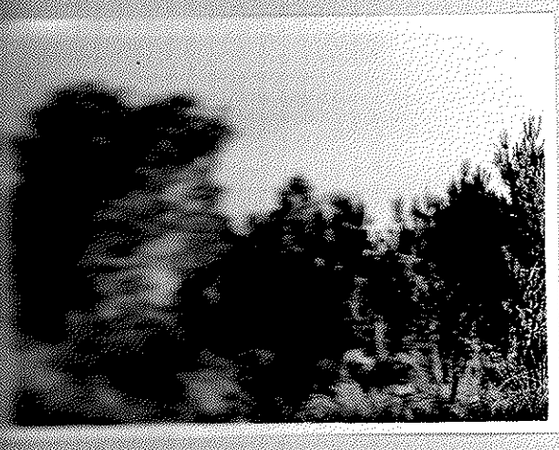
Clearfelled. Replanted South African 1938. Vestige of previous crop in background.

Ludlow C.17.

Mediterranean esterel or lucca.

Ludlow Compartment 10.

Poor atlantic; Landes.



Ludlow Compartments 12 & 13.

C.12 Mediterranean lucca broadcast 1920. C.13 seedlings from C.19 planted 1921. Refilled by broadcast sowing burnt patches 1929. Resowing 1929 to right atlantic landes.

Gnangara.

First and fourth trees from left Atlantic (Landes), others esterel.

Gnangara.

As for previous photo includes further esterel on the left.

Gnangara Headquarters.

Mediterranean esterel.
Occasional landes over topping.

Gnangara Headquarters.

Atlantic Landes.

Gnangara Headquarters.

Foreground Mediterranean lucca
20 years old.

As for previous photo.

Gnangara headquarters.
Another poor lucca, 20 yrs. old.

Gnangara, Compartment 19.

Left Atlantic leiria. Right Mediterranean corsican. Both 9 years old. Difference in height 5 ft.

Gnangara, Compartment 19.

Esterel left. Leiria right. Both 9 years old, difference in height 7 ft.

Gnangara, Compartment 19.

Landes left, esterel right. Both 9 years old, difference in height 5½ ft.

Perry has stated that the Leiria strain has shown the most desirable characteristics under Western Australian environmental conditions on the areas at present planted with the species. These areas consist of Eolian and Lateritic sands in the higher rainfall areas, i.e. 30" and over. However, attempts at acclimatisation in the drier areas at present under way or contemplated could possibly modify this view.

Duff (3) has given evidence of modification of form due to environment and Stoate (4) has shown examples of extreme stagnation and disguisal of form encountered in his work on nutritional disorders of Pinus spp. in Western Australia.

Mlle. V. Fieschi (5) has endeavoured to show specific differences between the so-called Atlantic and Mediterranean races on the grounds of needle structure differences and indeed has gone so far as to apply two names, Pinus pinaster and Pinus mesogeensis, respectively, on the somewhat slender evidence of number and placement of resin ducts. Perry is in the process of preparing a paper on this aspect so that further discussion here is precluded.

What does emerge from all this, however, is the possibility of ploidy, fixation or other nuclear differences and the first aspect is specifically dealt with in this study, while the question of raising plants for future plantings from seed in mixed plantations is one needing attention. This is cursorily dealt with pending detailed study.

Details of Procedure and Technique.

General.

1. In January 1950 an attempt was made to obtain mitotic counts from needle primaries of lateral leading shoots. The long chromosomes, however, made this difficult. It was of interest to note that division was completed at 6 a.m. and did not commence until after 1 a.m., that is, prophase was commencing between 1 and 2 a.m. and telephase was complete shortly after 6 a.m. Further work was deferred until the flowering period when it was anticipated that counts could be satisfactorily obtained.

2. In August the first male material was inspected and after certain difficulties had been overcome, satisfactory counts were obtained.

Technique.

Squashes of pollen mother cells were made using iron aceto carmine, but a heavy accumulation of protein granules in the cytoplasm completely obscured the chromosome figure necessitating rather drastic modification of the normal procedure as follows:-

1. Material was fixed in 3:1 acetic alcohol and after a period of 30 mintues it was placed in iron aceto carmine, lightly macerated, mounted and gently warmed.
2. Inspection of material was then made for evidence of division.
3. If division was taking place, the following procedure was carried out:-

   (a) Material was fairly strongly heated (not boiled)
   (b) Heavy pressure was applied
   (c) Further stain was added if considered necessary

The reason for this was that the cell walls were burst and in a few cases the chromosomes became separated from the cytoplasm and were readily seen. Much material was destroyed in the process, but satisfactory figures were obtained. (The use of a proteolitic enzyme is being tested to overcome this difficulty).

Stage of Meiosis found most satisfactory.

Due to the larger amount of dividing material, second division of meiosis was the obvious choice in view of the difficulties enumerated above and anaphase was the best period to obtain reasonable separation.

Types of Figure.

Polar view of second anaphase was aimed at and an occasional tetrad was obtained showing one or more separate haploid sets of chromosomes. The length of the chromosomes again was a difficulty and a great deal of material had to be inspected before a suitable image was found due to overlapping. Patience and persistence is required at this stage and four or five slides should be prepared at once to avoid repetitive delays.

Results.

The following table gives details of the counts made and the flowering dates. Numbers in the body of the table are haploid counts and notes refer to the state of the pollen mother cells.

Date	Strain, Locality and Flowering Notes.					
	C.10 Leiria	S.26 Esterel	S.27 Corsican	S.9 Landes	S Landes	G.H.Q. Lucca
8.8.50	12	Mitosis	Mitosis	Mitosis	Mitosis	Mitosis
17.8.50	Pollen	"	"	"	"	"
20.8.50	Shedding	Resting	"	"	"	"
28.8.50	"	12	12x	12	12x	12
5.9.50	"	Pollen	Resting	Pollen	Resting	Pollen
23.9.50			12		12	
27.9.50			Pollen		Pollen	

x Counts from aberrated conelets (see explanatory notes below)

C = Collier Plantation, South Perth
S = Somerville Plantation, Applecross
G = Gnangara Plantation, Caversham
HQ = Headquarters

Numbers indicate compartments.

A count was made of Pinus radiata ex Margaret River Plantation and this was also 12.

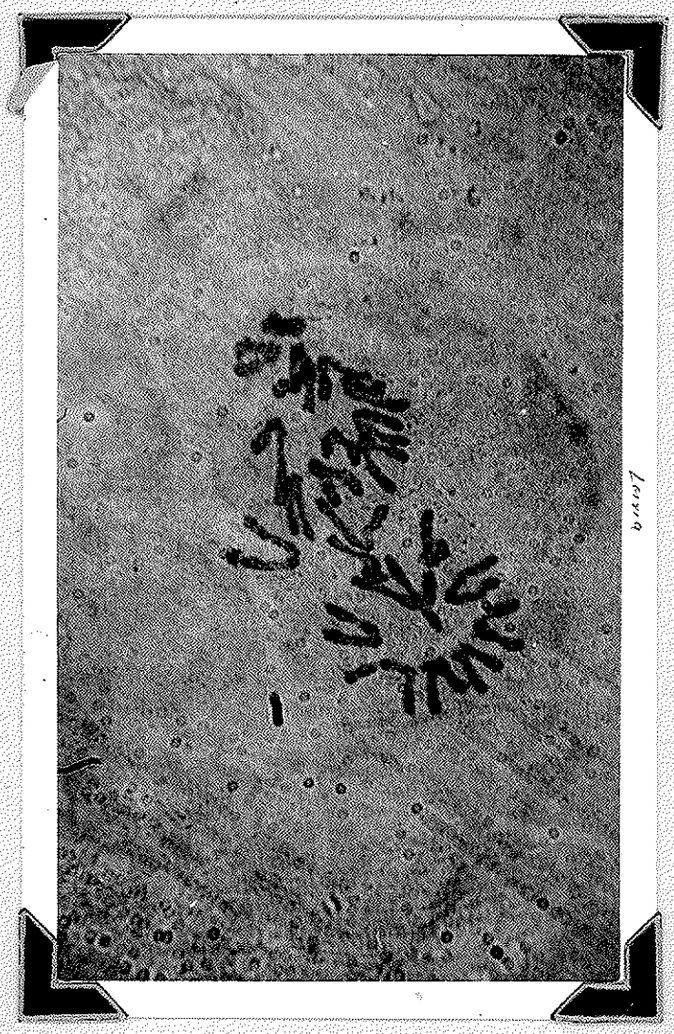
Observations on Meiosis.

1. The development of sporoginous tissue is followed by a resting stage and then the process of meiosis proceeds fairly rapidly. Differences in stages of individual trees allow a degree of latitude in obtaining material at the right stage, while storage in water if too early is often quite effective.

2. In all cases the meiotic process was normal and appeared uniform for all strains.

Chromosome figures.

The following microphotographs illustrate remarks made above and give some idea of the appearance of chromosomes of the species:-

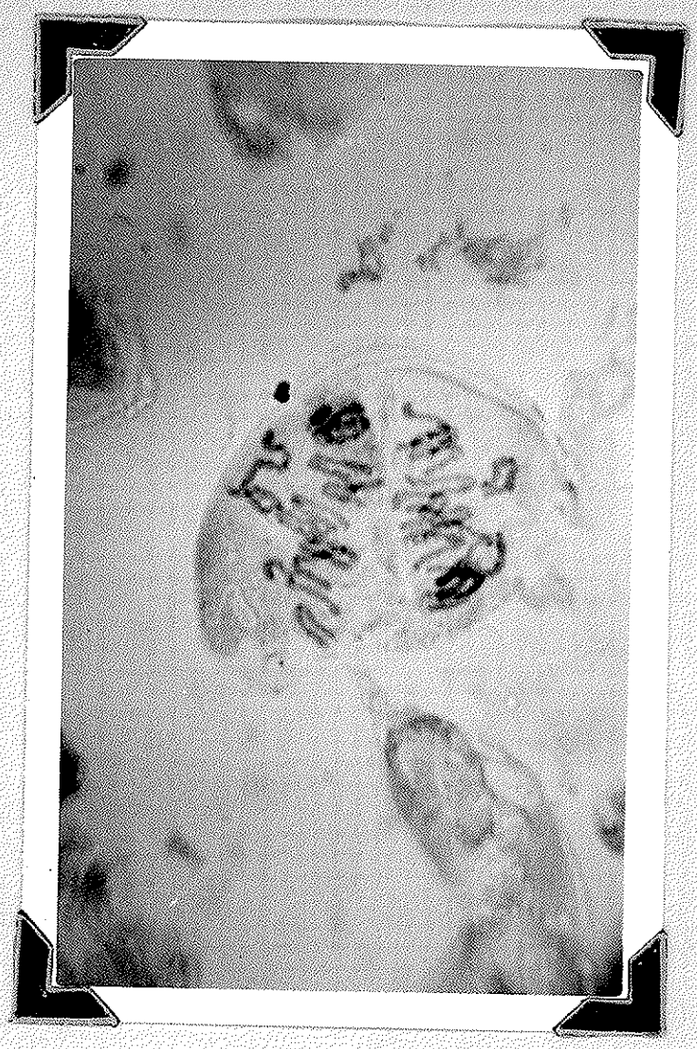


Leiria

Polar view, 2nd anaphase,
count x = 12.

Landes

1st series. Longitudinal 2nd anaphase. Chromosomes in collapsed microsphore mother cell. Tetrad cell walls showing. Count, x = 12.

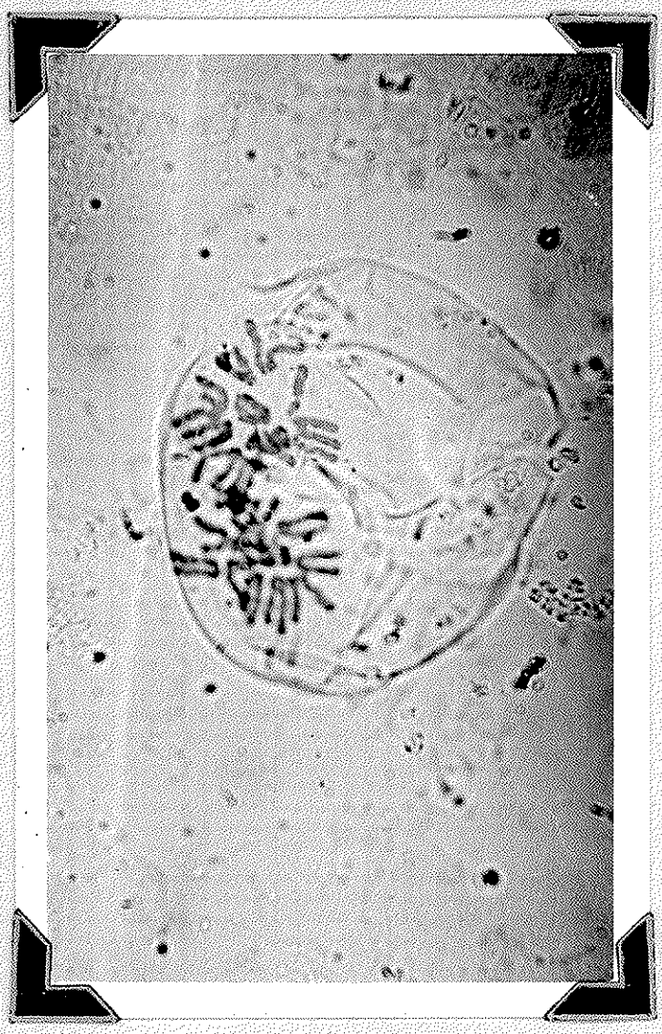


Landes

Series 2. Similar to series 1. Different mother cell. Count, x = 12.

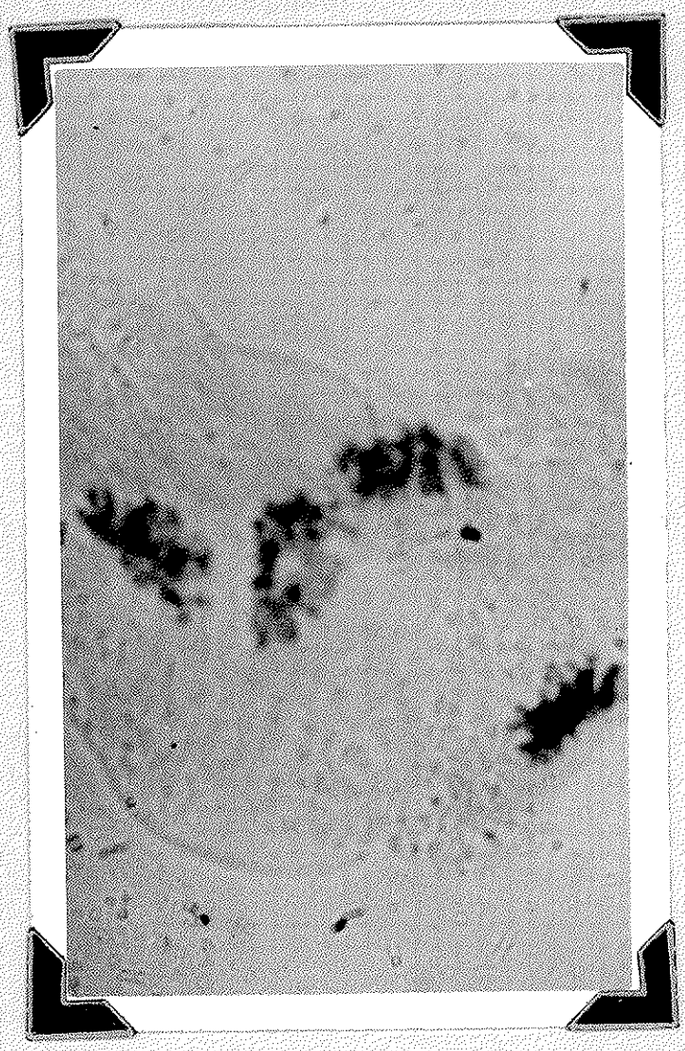
Lucca

2nd anaphase. Polar view. Count, x=12. (Unfortunately this slide dried out while camera was being obtained).



Esterel

Polar view, 2nd anaphase showing collapse microspore mother cell and tetrad cell walls. Count, x = 12.

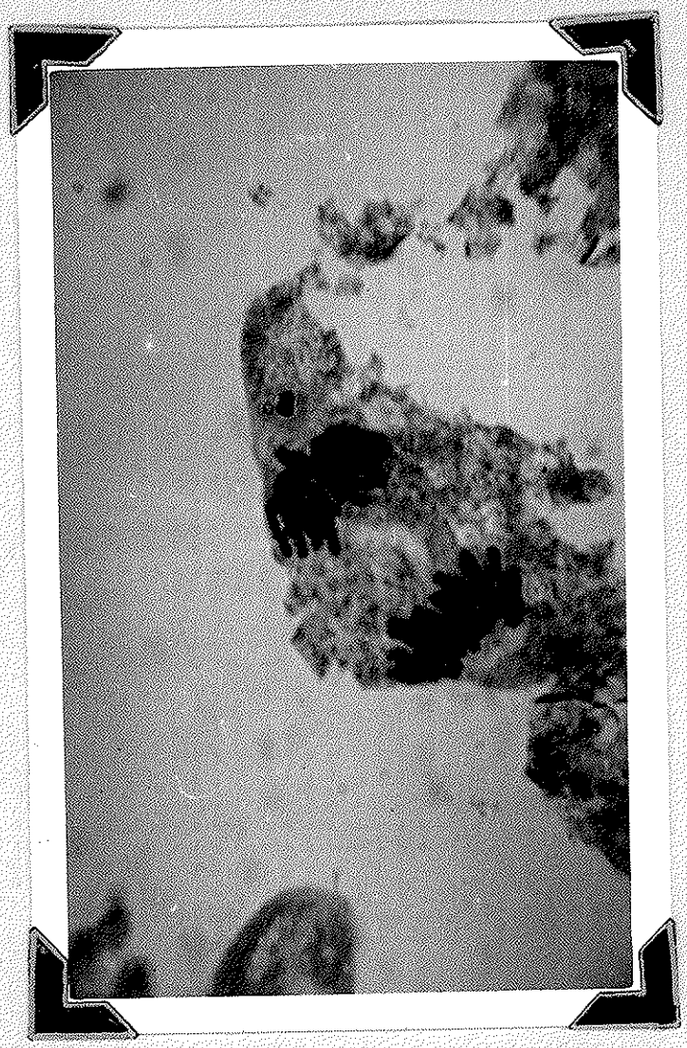


Landes

Showing longitudinals of chromosomes from the 4 tetrads. Ghost of microspore mother cell in background, dense cytoplasm below chromosome figures.

Landes

Similar to previous one. 1 tetrad chromosome compliment has been lost.



Landes

Longitudinal view of pair of tetrads at 2nd anaphase showing normal division behaviour.

Production of Male Catkins.

Pinus pinaster male catkins are slightly sub-terminal on the laterial branches, that is, a short leading bud is present to maintain the growth of the branch and the catkins are thickly clustered just behind this and leave a bare patch on the branch when shed. Scale leaves cover the catkins until pollen is ripening when the growth of the conelets bursts the scales and the conelets emerge, turn a reddish yellow or bright yellow and then shed their pollen.

It is of interest to note, however, that certain aberrations occur outside what may be termed the "grand flowering period". Specific instances were noted in Landes, Corsican, Esterel and Lucca strains where a few male conelets appeared to be in continuous production. This phenomenon was confined to the lower branches and may be due to physiological upsets of an unknown nature. However this production of apparently viable pollen does go on and the extent of hybridisation could be more extensive than previously anticipated; that is, if hybridisation does occur.

Production of Flowering Material on very young trees.

In view of the accepted policy in Western Australia of adhering solely to the Leiria strain, caution must be exercised until progeny trials have established the degree of purity of stock from local seed.

Male conelets have been observed on Leiria strain trees in the second year after planting out of 1/0 nursery stock and female cones are not uncommon in the third year.

In such case catkins are usually not formed, but a mass of conelets occur either thickly clustered or widely separated over a considerable portion of one or more lateral shoots.

Normal cone production does not occur until the fifth year or even later.

Possibilities of hybridisation and implications regarding stock raised from locally collected seed.

Although the cytological complexes of the strains of P. pinaster are not fully known, it has been established that ploidy does not exist and that flowering processes are normal. It is, therefore, probable that crossing takes place and until definite proof for or against this is obtained, it must be assumed that hybridisation occurs to a reasonable degree.

It must be admitted that Leiria strain flowers early, but on the other hand, there is a spread or overlap and it is known that female cones have been produced as late as March and male conelets of other strains are producing pollen in the leiria "grand flowering period".

Some variations in first generation stock from local plantation seed has been noted. This occurrence may be due to hybridisation or may be attributable to other causes not pertinent to this report. It, therefore, appears desirable to confine seed collection to trees raised from imported seed until investigation has progressed further into the hybridisation aspect and the type of progeny which might be expected after the first generation hybrid.

These and other problems are listed in the current research programme of the Forests Department.

✗ In 1948, 6 of 30 observational trees produced new cones after September, and in 1949 young cones were observed as late as March on trees affected by "twisting disease" (4).

References quoted.

(1) Perry, D.H. "Pinus pinaster in Western Australia", Australian Forestry, Vol. V, No. 2, 1940.

(2) " " "Pinus pinaster in Western Australia", Australian Forestry Conference, 1949.

(3) Duff, C.E. "The varieties and geographical forms of Pinus pinaster". British Empire Forestry Conference, 1928.

(4) Stoate, T.N. "Pine Nutrition". Forests Department, W.A., Bulletin 50.

(5) Fieschi, V. "Anatomie de la Feuille chez. les Pins Maritimes", Bull. Soc. d'Histoire Nat. de Toulouse, Vol. XIV, (1932).

Other references.

Burbidge, Nancy "Root development in Pinus pinaster and the Seasonal Variation of its mycorrhiza", Aust. Forestry, Vol. I.

Perry, D.H. "The effect of superphosphate on Pinus pinaster", Aust. Forestry, Vol. IV, No. 1.

Bednall, B.H. "A soil survey of Wyalup Plantation (P. Pinaster) area", Aust. Forestry, Vol. V, No. 11.

Rycroft, H.B., Wicht, C.L. "Field trials of geographical races of Pinus pinaster in South Africa", Fifth British Empire Conference, 1947.

Dellimore, W. & Bruce Jackson, A. "A Handbook of Coniferae".

Shaw, G.R. "The Genus Pinus".

Chamberlain, C.J. "Gymnosperms, Structure and Evolution".

5.10.1950

APPENDIX II

ANNAES III

INDUÇÃO DE POLYPLOIDIA EM PINUS PINASTER SOL. E EM QUERCUS SUBER L.

(The Induction of Polyploidy in Pinus Pinaster Sol. and Quercus Suber L.)

by

ANTONIO PEREIRA MACHADO
Instituto Superior de Agronomia.

Separata from Anais do Instituto Superior de Agronomia Lisbon Vol. 14, 1943.

translated

by

E.J. PIRIE,
COMMONWEALTH FORESTRY BUREAU
OXFORD.

October 1950.

THE INDUCTION OF POLYPLOIDY IN PINUS PINASTER SOL. AND QUERCUS SUBER L.

By: Domingos Pereira Machado
Instituto Superior de Agronomia.

Separate from Anais do Instituto Superior de Agronomia. Lisbon Vol. 18, 1949.

In the year 1939 we took over from among the projects of the Cork Oak Forest Experiment Station for the improvement of Quercus suber the section concerned with hybridization and induced mutation.

In dealing with the latter aspect of the problem we tried to obtain tetraploid forms by means of aqueous solutions of colchicine, and alkaloid which, since the work of Blakeslee and Avery (J. Hered. 28, 1937 (393-411)) has been widely used in the induction of polyploidy.

As the results obtained have been unequal we resolved in this year to produce similar changes in Pinus pinaster at the Institute Forest Laboratory.

Polyploidy, as is already known, in addition to the improvements it can bring about in obtaining new forms which may be immune to certain diseases, or may be more vigorous, more fertile and of more rapid growth etc., is almost always accompanied by gigantism, a character which is not to be despised in view of the fact that timber is the main product of P. pinaster.

In 1939 we used the three following kinds of treatment:

1. That recommended by A. Levan :- a drop of 1% colchicine or agar-agar jelly containing 1% colchicine is placed upon the eye between the cotyledons of very young plants twice a day for 10 days.

2. The aerial parts of young plants, not hitherto exposed to the action of the alkaloid are immersed in a 1% aqueous solution of colchicine for 1, 2 and 3 days.

3) Germination of seeds in cotton wool soaked in colchicine solutions.

By the first two kinds of treatment the development of the aerial parts of the plants was inhibited for some time; moreover the greater proportion of the plants died, and those that survived showed no signs of polyploidy.

We obtained better results however with the germination of seeds in Petri dishes with cotton wool soaked in aqueous solutions of colchicine. We used 1%, 0.5%, 0.2% and 0.1% solutions.

For each one of these solutions we used four series of seeds, which were in contact with the colchicine for 5,10,15 and 20 days respectively. When they had begun to germinate, and after being washed in distilled water, the seedlings were immediately placed in pots of soil.

The *L. suber* seeds of the series which were subjected to 20 days' treatment with 0.1% solution produced roots of much greater thickness than the controls (Phot. 1). By cutting some of these roots we succeeded in observing some cells which were bigger than the others, with very large nuclei and also some markedly irregular mitotic figures. This series of seeds was afterwards planted out in pots in suitable soil. Development was very slow in comparison with the controls, yet the seedlings showed no characteristics which distinguished them from untreated plants.

In 1939 we also tried to produce cytological changes in *L. pinaster*. Two series of seeds were placed to germinate, 1. in water, and 2. in an 0.1% aqueous solution of colchicine. As soon as the roots began to show, the seeds were washed and planted in pots containing soil. Most of the treated seeds showed thicker roots in which, when submitted to a cytological investigation, we observed, mainly toward the periphery, some cells in the process of division with a number of chromosomes exceeding 24.

Some of these seeds developed into plants with thicker stems and shorter and thicker needles than seedlings developing from untreated seeds (Phot. 2). These fleshy-looking seedlings had all the

the appearance of tetraploidy. By a comparison of the size of the stomata we observed that these were larger in the plants grown from treated seeds.

These plants were treated with the greatest of care, but unfortunately they all died. The 0.1% solution of colchicine in which the seeds were placed to germinate must have had a lethal effect and the time during which the alkaloid acted upon the embryo must have been excessive.

This year in the Institute we determined to carry out a small-scale experiment with seeds of P. pinaster only, but lowering the concentration of the solution and decreasing the duration of the treatment. We used an 0.05% colchicine solution, and, as soon as the seed coat gave signs of splitting to allow the root to emerge, the seeds were put to finish germination in pots containing soil. There survived only two plants with characteristics of tetraploidy- very thick roots and very short and thick cotyledons; these two plants also died after a very short time.

By a cytological examination of one of these thicker roots we noted extensive zones of very large cells with big nuclei, and, in some in the process of division we saw that the number of chromosomes exceeded 24 (Microphot. 1) and, in spite of the fact that in Coniferae the chromosomes aredifficult to count because many of them bunch together in the centre of the plate, we are convinced that they were 48 in number and therefore tetraploid.

We intend to continue the experiments next spring, but on a bigger scale, using 0.05% and 0.025% solutions of colchicine, and in view of the fact that P.pinaster appears to be very sensitive to the action of the alkaloid, reducing still further the duration of the treatment.

Sub-titles to the figures.

PHOT.1. Cork Oak seeds treated with 0.1% colchicine. Note the greater thickness of the roots compared with that of the untreated seed.

PHOT. 2. The Pine on the left, having grown from a seed treated with 0.1% colchicine has a thicker stem and shorter and thicker

leaves than that on the right, which grew from an untreated seed.

PHOTOT.1.

Metaphase in the root of Z.elegans, the seed of which was treated with 0.05% colchicine. The chromosomes are bunched together in the centre of the plate and their exact number is difficult to determine. However we believe that they number 48.

APPENDIX III.

Appendix III

STIMULATION OF GERMINATION PINUS PINASTER (LEIRIA.)

Of all the strains of P.pinaster, the Portuguese has always shown a semi dormancy which results in an extraordinarily long period of germination. Steady germination in trials, under conditions of continued moisture application, has been spread over as much as 18 weeks which is much too long.

Promising indications have been obtained by stratification which involves low temperature treatment of moist seed.-

1. Approximately 12% moisture by weight is added to dry seed.(This actually corresponds to thorough wetting of seed by immersion in towelling.)
2. Towelling parcels are then placed on racks from which drainage is free.
3. Temperature is reduced to 34 deg. to 37 deg.F and kept steady.
4. Treatment is continued for 3 - 6 weeks depending on species.
5. The outer surface of the towelling is used as an indicator of moisture level. If drying is too severe futher water may be added.
6. Seed is unwrapped after treatment and placed in jute or hemp transit bags. No apparent harm is done if seed if kept for a considerable period after treatment, but it is better to avoid such storage and sow as soon as possible after treatment.
(Re-stored seed has been tested after 6 months with 50% germination which was as high as the original rate.)

This treatment, apart from the cold aspect which may be part of the germination phenomena, ensures thorough wetting under controlled conditions and for this purpose trials have included wetting treatments.

It was thought that a detergent such as Teepol might be of assistance in this regard and in this years trial 5% concentrations were tried in addition to normal tap water treatments. The P.H. valve for tap water used was 7.0 and for 5% Teepol was 7.5

Three series ofexperiments were carried out at Collier Plantation, 4" deeppinus boxes being used, each box carrying one replication as far as practicable, but, where an overflow did occur, the nextbox was placed immediately adjacent to its major replication centre. All boxes were left in the open to approximate field conditions. Drying out was avoided however.

The aim was to try mid winter, late winter and early spring sowing periods in relation to the various treatments. Three seriels of seed were used. 685 Somerville 1948-49, 726 Somerville 1949-50, 750 ex Portugal 1950.

Experimental details were:-

Series. 1. Stratification v Teepol, water soaking abbresion.
Expts. 1A, 1B and 1C.

Treatments.

1. Stratification 3 weeks.
2. Control.
3. Soaking in cold water 24 hours.
4. Soaking in 5% Teepol 24 hours.
5. Soaking in 5% Teepol 12 hours.
6. Abbresion (rubbing on glass paper.)
7. Soaking in cold water 12 hours.

Sowing dates were:-

1A, X2 21.5.50.
1B, 11.7.50
1C 2.8.50.

Stretification dates were:-

Start 26.5.50 Finish 13.7.50
" 17.6.50 " 11.7.50
" 12.7.50 " 2.8.50.

X1. This was done in refrigeration at Botany Dept. and control was not complete. Freezing occurred in each series.
X2. Stretification was delayed and sown late - not quite 3 weeks and not quite comperable to remaining treatment but has been included for uniformity of treatments.

Series. 2. Blanket Hormone treatment v water soaking.
Expts. 2A, 2B and 2C.

Treatments.

1 Control.
2. Soaked in Formula 2o (Kerners) 12 hours.
3. Soaked in Formula 20 " 24 hours.
4. Soaked in cold water 12 hours.
5. Soaked in cold water 24 hours.

Cold soakings give a basis for contrast with series 1.

Sowing dates were:-

2A. 12.6.50
2B. 11.7.50
2C. 2.8.50.

Series 3. Stretification v Teepol ½ and ¼ and water soaking.
This was a check series as the bulk stretification sent out for sowing in 1950. Stretification was arranged to meet sowing dates of 25.7.50 and 1.8.50 at Dwellingup and Busselton, Divisions respectively. This involved Serials 726 and 750 only.
Expts. 3A and 3B.

Treatments.

1. Unstretified.
2. Stretified 3 weeks.
3. Soaking in ½% Teepol 4 hours } unstretified seed.
4. Soaking in ¼% Teepol 4 hours }
5. Soaking in cold water 4 hours. }

Note. The soaking time was reduced in view of poor results with longer teepol treatment and was a late modification of soaking in view of the 12 hour soaking results.

Sowing dates were:-

3A 25.7.50
3B 1.8.50

Stretification dates were:-

Start 28.6.50 Finish 18.7.50
" 4.7.50 " 25.7.50.

Summary of Analysis.

Reductions were made for 6 weeks and 8 weeks.

Series 1. Unit 30.

(a) Simple analyses. Serial numbers separate.
(b) Seasonal analyses. Serial numbers separate.
(c) Split plot combining all serial numbers.

Serial 2.

As per Serial 1.

Series 1.

(a) Simple analyses serial numbers seperate.
(b) Split plot combining all serial numbers.

x. These analyses will be done at a later date.

Series 1. Details.

A. Simple Analyses.

(a) 6 weeks.

Serial 685.

Treatment.	Means. 1A.	1B.	1C.
1	2.0	7.2	14.6
2	2.6.	1.4	6.4
3	4.4	3.8	3.4
4	.6	0.0	1.4
5	0.0	2.2	4.6
6	2.8	3.6	9.0
7	4.8	4.4	9.4
Gen. Means.	2.45	3.22	6.93
SEDM	1.26	1.21	1.57
Signif-icent.	.01	.01	.01
Diff. .05	2.60	2.50	3.24
Diff .01	3.52	3.38	4.39

(b) 8 weeks.

Serial 685.

Means. 1A.	1B.	1C.
5.2	8.o.	16.8.
3.2	1.4	11.8
5.2	4.0	5.6
.6	.2	2.8
.2	3.0	7.8
3.6	3.6	14.6
5.2	4.4	14.0.
3.31	3.51	10.46
1.49	1.54	1.85
.01	.01	.01.
3.08	3.18	3.82
4.17	4.31	5.17

Results.

(a) 1A. No differences from Control.
1B. Stretification better than all others except soaking 12 hours $P > .01$ but better than that treatment at the .05 level. No other treatments better than control.
1C. Stratification better than all other treatments $P > .01$. No other treatments better than them Control.

(b) 1A. No differences from Control.
1B. Stretification bettern than all other except Soaking 12 hours $P > .01$ but better than that treatment at the .05 level. No other treatments better than control.
1C. Stretification better than control $P > .05$ approaches significence $P > .01$. There were no other differences from Control.

(a) 6 weeks.

Serial 726.

Treatment.	Means. 1A.	1B.	1C.
1	1.4	9.6	6.8
2	4.2	2.6	4.4
3	4.6	1.2	3.2
4	3.0	.4	1.8
5	1.4	2.6	3.8
6	3.4	.8	6.2
7	6.4	3.6	6.6
Gen. Means	3.46	2.95	4.66
SEDM	1.19	.91	1.36
Signif-icent.	.01	.01	.01
Diff. 2.05	2.45	1.87	2.81
Diff. .01	3.32	2.54	3.80

(b) 8 weeks.

Serial 726.

Means. 1A.	1B.	1C.
6.2	10.0	11.0
5.6	3.0	8.6
5.2	1.4	9.6
4.2	.8	6.0
2.2	2.6	7.4
3.4	1.4	10.6
7.0	4.6	10.6
4.82	3.4	9.11
.63	.97	.01
.01	.01	.01
1.30	2.00	2.76
1.76	2.71	3.74

Results.

(a) 1A. To treatment better than control.
1B. Stratification better than all other treatments P = .01. No others exceeded control.
1C. No treatment better than control.

(b) 1A. Control exceeded by soaking 12 hours P = .05 only.
1B. Stratification better than all other treatments P = .01. No others exceeded control.
1C. No treatment better than control.

(a) 6 weeks.

Serial 750

Treatment.	Means. 1A	1B	1C
1	0.8	5.6	10.8
2	2.4	2.2	8.4
3	5.2	6.4	7.0
4	0.2	0.0	1.4
5	0.0	2.6	4.8
6	3.2	3.2	7.8
7	5.4	5.6	7.4
Gen. Means.	2.44	3.64	6.8
Signif-icent.	.01	.01	.01
SEDM	1.11	1.46	1.67

(b) 8 weeks.

Serial 750

Treatment.	Means. 1A	1B	1C
	7.4	6.6	16.2
	3.6	3.8	10.6
	6.8	7.4	10.6
	0.4	0.0	4.2
	0.2	3.2	8.0
	4.6	3.8	10.8
	6.4	6.2	10.2
	4.1	4.3	10.06
	.01	.01	.01
	1.21	1.41	1.77

Diff .05	2.29	3.01	3.44	2.49	2.92	3.65
Diff .01	3.11	4.08	4.67	3.36	3.95	4.95.

Results.

(a) 1A Cold water soakings better than control P >.05. No other exceeded control.
1B. Stratification and cold soakings better than Control P >.05. No other exceeded Control P> .01 in case of 24 hours soaking.
1C. No treatment better than control.

(b) 1A. Stratification and Soaking 24 hours better than Control P > .05. Soaking 12 hours better than Control P >.05.
1B. No treatment exceeded control.
1C. Stratification better than Control of P >.01. No other treatments exceeded Control.

B. SEASONAL ANALYSIS. 1A plus 1B plus 1C.

(a) 6 weeks.

Serial 685

Treatments & Means.		Period & Means.	
1.	7.9	1	2.46
2	3.4	2	3.22
3	3.86	3	6.96
4	.66		
5	2.26		
6	5.1		
7	6.2		
Significant			.05
SEDM			1.49
P.05			3.24
P.01			-

(b) 8 weeks.

Serial 685.

Treatment & Means.	Period & Means.	
10.0	1	3.31
5.46	2	3.51
4.93	3	10.49
1.2		
3.66		
7.26		
7.86		.02
.05		.01
1.91		1.25
4.16		2.72
-		3.82.

Results.

(a) Treatments did not differ but period 3 was better than 1 and 2.

(b) Stratification was better than control. No other treatments differed from Control. Period 3 was better than 1 and 2.

(a) 6 weeks.
726

Treatment & Means.		Period & Means.	
1	5.83	1	3.48
2	3.73	2	2.97
3	3.00	3	4.68
4	1.73		
5	2.60		
6	3.46		
7	5.53		
Significent		-	
SNDM	-	-	
P.05	-	-	
P.01	-	-	

(b) 8 weeks.
726.

Treatment & Means.		Period & Means.	
	9.06	1	4.82
	5.73	2	3.40
	5.40	3	9.14
	3.66		
	4.13		
	5.13		
	7.40		
	.05		.01
	1.37		.90
	2.98		1.97
	-		2.76

Results.

(a) No effect of treatment on period.
(b) Stratification was better than Control but no other treatments differ from Control. Period 3 was better than 1 and 2.

(a) 6 weeks.
Serial 750

Treatment & Means		Period & Means.	
1	5.73	1	2.45
2	4.33	2	3.65
3	5.20	3.	6.8
4	.53		
5	2.46		
6	4.73		
7	6.13		
Signif-icent	.05		.01
SNDM	1.42		.93
P.05	3.09		2.03
P.01	-		2.84

(b) 8 weeks.
Serial 760.

Treatment & Means.		Period & Means.	
	10.06	1	4.14
	5.86	2	4.42
	8.33	3	10.11
	1.53		
	3.80		
	6.40		
	7.60		
	.01		.01
	1.12		.71
	2.44		1.55
	3.42		2.17

Results.

(a) No treatment was better than Control. Period 3 was better than 1 and 2.
(b) Stratification was better than control. Period 3 was better than 1 and 2.

EXPT. 2. (Unit 50 in 2A and 30 in 2B and 2C.)

(A) Simple Analyses.

(a) 6 Weeks. Serial 685.

Treatments.	Means. 2A	2B	2C.
1	1.6	1.8	4.0
2	3.6	4.2	5.8
3	5.0	5.2	2.2
4	4.3	4.4	7.8
5	5.6	1.8	3.4
Gen. Mean.	4.07	3.48	4.64
Significant		-	-.01
SED^M	--	-	1.22
P.05	-	-	2.59
P.01	-	-	3.56

(b) 8 weeks. Serial 685.

Means. 2A.	2B.	2C.
7.6	2.4	5.6
9.6	4.6	7.4
8.3	5.4	3.4
7.0	5.0	9.4
10.3	2.4	4.4
8.6	3.96	6.04
-	-	.01
-	-	1.49
-	-	3.16
-	-	4.35

Result. Soaking 12 hours appears to be the only tendency towards stimulus.

(a) 6 weeks. Serial 726

Treatments.	Means. 2A	2B	2C
1	1.0	2.8	2.6
2	3.6	4.8	5.2
3	3.6	1.8	3.4
4	4.3	5.8	4.8
5	5.3	2.0	3.6
Gen. Means.	3.6	3.44	3.92
Significant	.05	.01	-
SEDM	.96	.87	-
P.05	2.21	2.01	-
P.01	-	2.92	-

(b) 8 weeks. Serial 726

Means. 2A	2B	2C.
4.6	3.2	5.0
5.6	5.4	7.0
7.3	2.2	5.4
7.0	6.0	6.4
6.6	2.8	4.4
6.26	3.88	5.64
-	.01	-
-	.84	-
-	1.94	-
-	2.82	-

Results. (a)2A. All soaking treatments better than control. No other difference.
2B. Soaking 12 hours in water was better than control. 12 hours was better than 24 hours soaking.
(b)2B. Soaking 12 hours in both water & hormone better than control. 12 hours soaking was better than 24 hours.

(a) 6 weeks. Serial 750

Treatment.	Means. 2A	2B	2C.
1	2.0	2.4	6.0
2	4.3	5.2	5.0
3	4.0	5.2	7.0
4	3.6	5.4	7.6
5	4.3	4.2	5.6
Gen. Means.	3.66	4.48	6.24
Significatn			
SEDM	-	-	-
P.05	-	-	-
P.01	-	-	-

(b) 8 weeks. Serial 750.

Means. 2A	2B.	2C.
4.3	3.6	7.8
8.0	5.6	6.2
7.0	5.6	8.6
5.6	5.8	8.8
7.6	4.4	8.6
6.53	5.0	8.0
-	-	-
-	-	-
-	-	-

Result. No effect of treatment.

SERIES 3. Simple Analyses. Unit 50.

(a) 6 weeks.

Treatments.	Means. 3A	3B
1	7.8	14.4
2	5.6	17.6
3	8.2	12.2
4	7.0	8.2
5	7.4	12.8
Gen. Means	7.2	13.0
Significant	-	.01
SEDM	-	1.9
P.05	-	4.03
P.01	-	5.55

(b) 8 weeks.

Means. 3A	3B.
13.8	21.6
14.8	29.6
12.2	19.2
13.4	14.6
14.2	21.4
13.88	21.28
-	.01
-	2.71
-	5.75
-	7.92

Results.

(a) 3B ½% Teepol was depressing but no other treatment varied from the control.

(b) 3B. Stratified was better than all others. ½% Teepol was still worsethen control .

(a) 6 weeks.

Treatments.	Serial 750 Means. 3A	3B.
1	9.0	18.2
2	8.4	17.8
3	4.4	10.2
4	7.4	10.6
5	8.8	16.8
Gen.Means	7.6	14.72
Significant	.05	.01
SMDM	1.5	2.02
P.05	3.18	4.28
P.01	-	5.89

(b) 8 weeks.

Serial 750. I Means. 3A	3B.
16.0	24.2
16.4	26.8
10.0	17.8
14.2	15.6
16.4	24.2
14.6	21.72
.01	.01
1.76	1.45
3.73	3.07
5.14	4.24

Results.

(a)3A No treatment exceeded the Control. ½% Teepol was depressing.
3B No treatment exceeded the Control ½ and ¼% Teepol were depressing.
(b)3A No treatment exceeded the Control ½% Teepol still depressing.
3B No treatment exceeded the Control ½ and ¼% Teepol were depressing.

General Conclusions. Series 1.

(A) Simple Analyses.

Stratification tended to be better than control all the way through with soaking for 12 and 24 hours fluctuating in the series. Abbrasion rarely gave good results, of course this treatment is difficult to control.
Teepol of ½% Concentration is very depressing to germination.

General Means.

(a) 6 weeks.

Expt.	Serial 685	726	750
1A	2.45	3.48	2.45
1B	3.22	2.97	3.65
1C	6.96	4.63	6.8

(b) 8 weeks.

Expt.	Serial. 685	726	750.
1A	3.31	4.82	4.14
1B	3.51	3.4	4.42
1C	10.49	9.14	10.11

The general trend was for germination rate to increase with late sowing.

B. The Seasonal Complex Analysis, showed(1) period 3 to be better than periods 1 and 2 in all cases. Periods 1 and 2 did not differ.

(2) There was no differentation from control at 6 weeks but at 8 weeks stratification was better than control in every case. Soaking 24 and 12 hours held an intermediate position with abbrasion fluctuating. Teepol was particularly depressing at the 24 hour soaking standard.

As might be expected, finer treatment differences are lost in this overall analysis due to extra variation introduced.

However, the position is fairly clear cut in favor of spring sowing with stratification as an additional aid.

Series 2. General Conclusions.

Hormone treatment was not effective. Soaking for 12 hours gave an impetus in serial 685 and 726. The incidence of effect was variable in relations to period but there was the same trend as in Series 1 for germination to be greater in period 3.
Serial 750 showed no effect except for the period trend.

Series 3.

Late sowing tended to be better than early sowing.
Teepol was depressing.
Stratification was an advantage with 726 after 8 weeks, but although well up in the other experiments, I only approached significance in 8 weeks with Serial 750.

SUMMARY OF RESULTS.

The locally collected seed, serials 685 and 726 appeared to respond to treatment differently to the imported Portuguese seed 750.

This ties in with the chemical test for viability.
Series 1 and 2 were the main tests and 3 was aimed at checking the germination of bulk stratification. Nursery indications check with Series 3 results on an observational basis.
Time of sowing appears to be important and the early spring period has been generally beneficial. Of course the prolonged cool, moist weather since August may be taken as a factor in favour of controls.
The actual wetting of seed does not appear to be the answer since low concentrations of Teepol have been depressing. This must be qualified by a possible toxicity of this detergent. However, survivals do not show signs of damage so perhaps some weight must be placed on possible over wetting.

Generally, it could be said that spring sowing is advantageous, while stratification is an aid. Cold water soaking appears to be helpful too. Further experimentation is indicated to test different seasonal weather.

Locally collected seed appears less stable than imported and it is considered that the units should be increased to 50.

In future trials, boxes will beeliminated in favor of germinating beds if this can be arranged and watering will be eliminated also if this can be arranged.