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GERMINATION IN PINUS PINASTER AIT.

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GERMINATION IN *Pinus pinaster* AIT

SUMMARY

Germination of seed of *Pinus pinaster* varies greatly in the nursery with seedlot, storage conditions, pretreatment and year of sowing. This bulletin reports studies carried out to define a standard testing technique which will provide for precision and reliability in nursery sowing.

Germination behaviour was found to vary with season, temperature, light, environmental preconditioning, stratification and genotype. The most suitable test procedure defined employs a seven day cold soak, three week stratification at 3°C and four week incubation at 15°C with 14 hours' light each day. Precision in testing is dependent on correct subsampling of seed batches and the provision of a light stimulus to the seed, during extraction from the cone.

To obtain maximum germination in the nursery, pretreated seed should be sown in August. Stratification greatly improves the energy of germination but does not necessarily increase total germination. Germination may be seriously reduced if the seed does not receive a light stimulus before sowing. Normally, exposure during extraction from the cone is adequate and surface drying of stratified seed in sunlight, prior to drilling, will overcome further light deficiencies.

Germination was seriously impaired at temperatures above 20°C and below 15°C. The most favourable test temperature was 15°C; no advantage being obtained with fluctuating temperature regimes.

Germination for this species is a most complex and sensitive process. Highly significant effects were associated with genotype and interactions between genotype and environment while viable seed was retained on the tree. The preconditioning effects are not understood but could influence the economy within seed orchard management.

GERMINATION IN *Pinus pinaster* AIT

I. THE PROBLEM

Introduction

Pinus Pinaster is a forest tree species of commercial value in southern Europe, northern Africa, South Africa, southern Australia and South America (Scott, 1962). Generally, seed has been plentiful and most artificial establishment involves direct sowing into the field.

In Western Australia, this exotic has been planted over 40,000 acres (16,000 ha) and extension proceeds at the rate of 2,000 to 4,000 acres (800 to 1600 ha) per annum to a probable target of 150,000 acres (61,000 ha). Within the State economy, the species has commercial value provided that establishment and maintenance costs are kept to a minimum. Machine planting of nursery raised seedlings has been found essential to these conditions.

* Note: Metric equivalents are given in the text but not in accompanying tables or appendices.

Problems are associated with seeding in cultural practice. David and Guerindon (1951) have shown that seed of *Pinus pinaster* is subject to a dormancy condition. Further work in Western Australia (Hopkins, 1960), Uruguay (Bonilla and Rava, 1963), South Africa (Donald, 1963) and France (David, 1962; Guitton, 1965) indicates that germination is considered to be a problem throughout the range of use of the species. From published work it appears that both seed coat inhibition and a natural dormancy process may influence practical results. Harding (1952) has also shown that mechanical treatment during winnowing may significantly influence germination.

In Western Australia periods of up to 12 weeks have been associated with germination of untreated seed. Total germination, measured over this period, has varied from 20 to 80 percent with seedlot and year. Pre-treatment by cold soaking and stratification (Hopkins, 1960) has effectively reduced the germination period within the nursery and improved uniformity in plant size. Percentage germination does however, still vary considerably with seed batch, length of storage, year of sowing and other unknown causes.

The local plantation programme for *Pinus pinaster* recently increased from 2,000 to 4,000 acres (800 to 1600 ha) per annum. This increase, coupled with a need to reduce nursery and establishment costs and to plan for seed of orchard origin (Perry and Hopkins, 1967), focussed further attention on seed performance. Recent problems stem more from the need for a satisfactory procedure to predict germination percentages obtainable in the nursery than from a desire to further increase the energy and total germination of any seed batch.

The present bulletin is an extension of work previously reported (Hopkins, 1960) and is concerned with factors which influence the germination of seed of *Pinus pinaster* of the Portuguese provenance. The report covers a scattered series of experiments, carried out over a period of years, with the objective of developing a standard testing technique effective in predicting the correct amount of seed to sow in the nursery. Interesting behavioural patterns or responses to temperature, light, seed maturity, genotype and environment are reported. These assist one to appreciate the extent of the problem associated with handling seeds of this species. A suitable technique for germination testing has also resulted.

This report does little towards exploring the physiology of germination in the species and it is hoped that its presentation may stimulate research in this field. Further explanation could lead to appreciable economies in improvement programmes conducted for the species in France, Portugal, South Africa and Australia.

Previous Testing Procedure

A reliable estimate of the germinative performance of any seed lot to be sown is essential for sound nursery practice. Without this estimate the rate of sowing cannot be regulated with any degree of certainty and spacing within rows may be too close or excessive. Extremes of spacing influence both seedling survival in the field and, within a large nursery programme, economy of nursery space. *Pinus pinaster* in Western Australia is sown in August with seed imported from Portugal. The germinative performance expected in August is predicted from tests carried out some time in the preceding six months.

The standard procedure used to determine germinative capacity has been to test batches in six inch (15 cm) pots, filled with sand and standing in a one inch (2.5 cm) depth of water. A clean sheet of glass is placed over the pot, the seeds being lightly covered with sand. All tests were carried out in the open and a nine week germination period was employed. It had been suggested that this procedure simulates nursery conditions and provides an estimate of the number of plants which will develop, rather than a value relating to seeds which will germinate to some extent but may not survive nursery conditions.

The technique is unwieldy and there is no reason why it should give a superior figure for germination than that of many other simpler, artificial procedures. David and Guerindon (1951) have recorded that the germination of *P. pinaster* seed varies with season. Since the local tests have to be carried out prior to the month of sowing, the test results may have little direct bearing on the seasonal result in August. Although operators conducting the standard testing at Como had commented that germination rate did appear to vary with the incidence of cloudy or clear weather, no monthly calibration had been carried out to ascertain the significance of seasonal variation to the test procedure.

Criticism of the technique led to the Seeds Branch carrying out monthly comparisions in the periods 1958-59 and 1960-61. Variability in performance with season was verified. In 1964 detailed studies into the germination behaviour of *P. pinaster* seed were initiated as background research to the tree improvement programme for the species. This work continued into 1969 and is considered to be completed from the current operational viewpoint.

(i) Tests of Standard Procedure

Monthly tests comparing the performance of five pots each containing 100 seeds of *P. pinaster* were carried out in the standard facilities at Como from October 1958 to September 1959 and August 1960 to July 1961. Different seed batches, each recently imported from Portugal, were used in each case. *Pinus radiata* and several other species were included in the trials for purposes of comparison. For each monthly unit, counts were made at weekly intervals for nine weeks. Results are expressed in Figure 1 to indicate germination for each monthly trial (commencing date) as a percentage of the highest value achieved during each twelve monthly period.

The maximum germination values obtained for *P. pinaster* in the 1958-59 and 1960-61 trials were 76 and 84 per cent., respectively. Minimum values were 15 and 21 per cent., respectively. It is obvious from these ranges that poor choice of the month for testing could lead to erroneous estimates of germination in August and to expensive results in practice.

From Figure 1 it can be seen that within a single year's testing, variation between months is great. Variation is also considerable between results obtained in any one month for the two years of testing.

The data for *P. radiata* are included in the figure in an effort to separate seasonal effects from species trends. For *P. radiata* the general level of germination is higher (maximum for 1958-59 and 1960-61 being 83 and 88 per cent. respectively) and more even. The differences between summer and winter performance for both species follow a similar pattern.

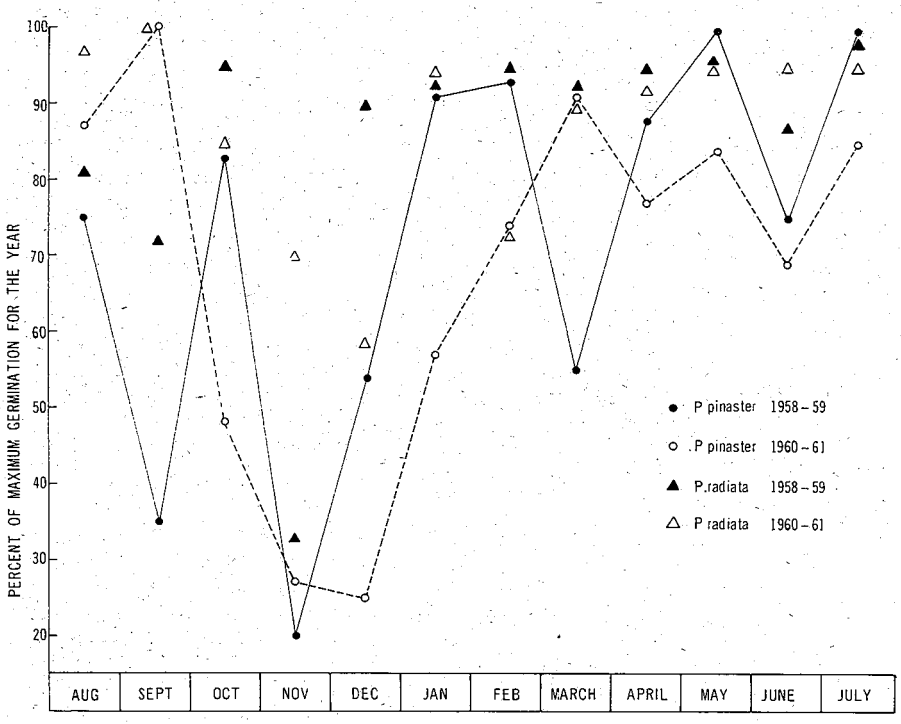

Figure 1.
Variability in germination obtained by sowing at monthly intervals in the open. Results for both *P. pinaster* and *P. radiata* are compared over two separate twelve month periods.

Results revealed that monthly germination may depend on species, seed batch and ambient conditions (Figure 1). From the data it appears safe to say that the best time to test *P. pinaster* with the standard technique is in April-May and July-August. The poorest time, which was for both species and is hence probably associated with ambient conditions, is during the months October to January. Assuming that test conditions approximate nursery conditions the trial confirms the fact that July-August is the best time for nursery sowing in the Perth Metropolitan region.

TABLE 1

Ratio (per cent) of Germination at Four Weeks to Germination at Nine Weeks for Seed Tested Monthly in Open Beds in Different Years. Seed Batches Used in Separate Years are Different.

Year of Comparison	Month											
	A	S	O	N	D	J	F	M	A	M	J	J
1958–1959....	77	89	75	33	35	42	47	26	67	84	80	82
1960–1961....	37	88	60	96	86	46	48	70	58	63	55	19

In Table 1, the ratio of germination at four weeks to that at nine weeks is calculated to compare the influence of season on germinative energy. Agreement between years for any month is poor. Of the 24 monthly values only nine provide more than 70 per cent. of the total germination (recorded for that month) in a four week period.

These data illustrate the slow and erratic behaviour of *P. pinaster* seed with season. The species is more difficult to evaluate than *P. radiata* and a serious adverse effect on germination is common to both species in early to mid summer. It was not possible to ascertain whether this adverse effect is of seed origin or due to ambient conditions. The latter would appear to be more logical since both species performed similarly under the conditions.

(ii) Tests on the Laboratory Bench

Preliminary investigations were limited by facilities available and aimed to define a reliable laboratory procedure for seed testing. While developing techniques, monthly performance within the laboratory was compared to verify previous results obtained under completely uncontrolled conditions.

Two separate seed batches, one collected and extracted locally and another imported from Portugal, were compared. Five replications of 100 seed units were germinated on filter paper in petri dishes, monthly, during 1965 and 1966. Results are expressed in Figures 2 and 3.

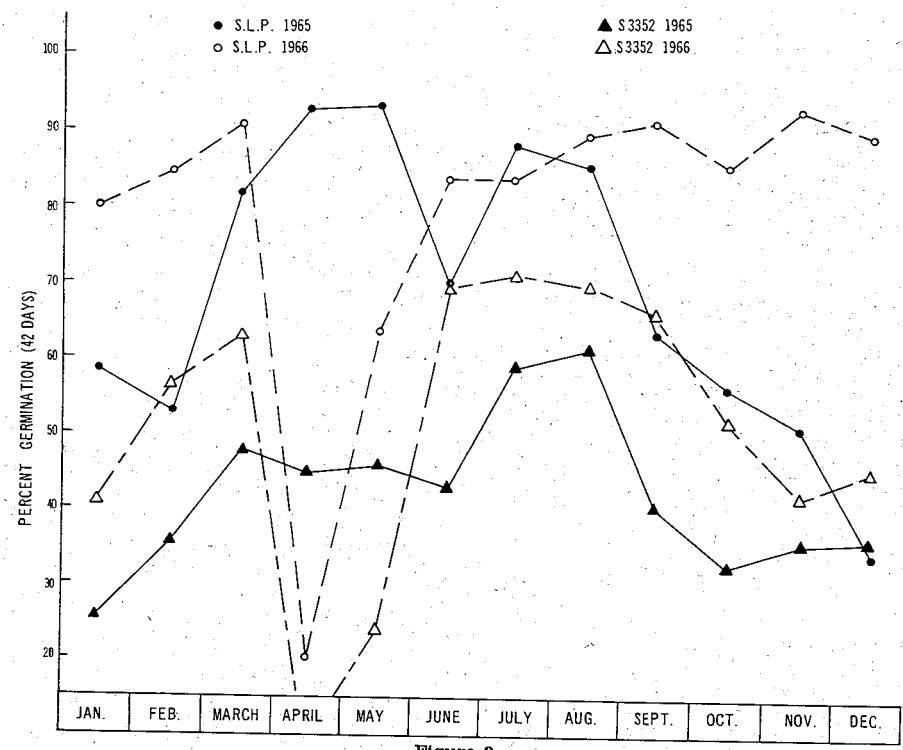

Figure 2.
Seasonal variation in two seed batches of *P. pinaster* germinated in the laboratory, on the bench, at monthly intervals for two consecutive years.

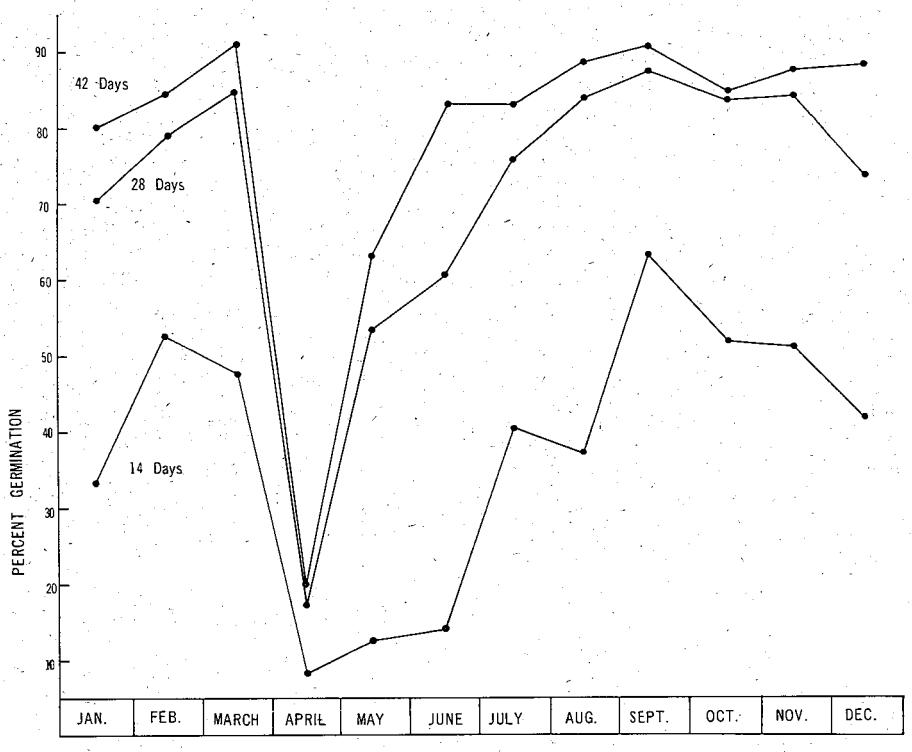

Figure 3.
Progression of germination by season for 14, 28 and 42 day germination periods. Tests were carried out in petri dishes on the laboratory bench.

The use of petri dishes in a fixed position on the laboratory bench provided some further degree of control to the testing system. It can be seen from Figure 2, however, that monthly variation was still present. It followed the same trend for each seed batch within any one year but there was little general relationship in the trends between years. Germination was best in most tests in July and August and was consistently poor in summer. The current trial provided completely different results for autumn each year revealing that, apart from the two periods mentioned above, monthly variation may be quite unpredictable.

In Figure 3 it is shown that germination within petri dishes follows a consistent pattern whether results are expressed for 14, 28 or 42 days. It is therefore possible to further studies using a 14-day germination interval, if trends are all that are required and if time for testing is a limiting factor.

Discussion

Prior to the present study, the complexity of germination behaviour in *Pinus pinaster* was not appreciated. The monthly variation under ambient conditions represents a factor which can seriously affect operational results. Early departmental work which separated August as the sowing date for this species in Western Australia reflects a sound appraisal of seed performance

under field conditions. Results from the current tests in open beds or on the bench (Figures 1, 2 and 3) suggest that delaying this sowing date by only one month in the Metropolitan area could lead to a serious deficiency in nursery plants.

The experimental programme which developed to define a precise and predictable germination technique is reported in sections concerning temperature, stratification, light and seed maturity and genotype. In fact, most experiments were factorials and considered two or more of these factors at any one time. To sort out the complexity found and to explain variable results, the sectional presentation was considered to be most desirable and practical.

Testing Technique

All further work reported followed a standard procedure. Seed batches used were maintained in cold storage at 4°C. Prior to testing, seed was soaked in water at room temperature for seven days, all floating seed being discarded. Where stratification is involved the soaked seed was placed in polythene bags and maintained at 3° to 4°C for the required period.

Prior to testing, all seed was surface dried either in the sun or in a forced draft at 30°C for four hours. It was then lightly dusted with Captan, a fungicide. A test unit consisted of 100 seeds on filter paper placed on an inverted watch glass contained in a 11.0 x 1.5 cm petri dish. Water was added to the dish as necessary and distributed over the filter paper by cotton wicks.

Counts were made at 7-day intervals, the seed with radicles developed to at least the width of the seed being removed from the dish and recorded. Results were expressed as percentages, transformed to angular arcsine and analysed for (a) 14 day, (b) 28 day germination and (c) germination energy ((a) divided by (b)).

Early studies were carried out on the laboratory bench or in standard incubators with no refrigerated control. Major studies were developed in a specially constructed incubator which could maintain temperatures within the range 5°C to 30°C. Twin circuits and a coupled time switch permit automatic control of any two temperature regimes between these limits. Lighting can be supplied by either fluorescent tubes (4 Mazda 40 watt daylight tubes at 18 inch distance) or incandescent lamps and can also be automatically controlled to two schedules within each 24 hours. The incubator can accommodate 72 units for any one trial and provide temperature control to within one degree centigrade.

For later studies, several refrigerated cabinets controlling fixed temperatures from 5°C to 30°C were also available for use.

II. THE INFLUENCE OF TEMPERATURE

Introduction

The object of the study was to develop a testing procedure, for seed of *Pinus pinaster,* which will provide precision of result for any month of testing. The result obtained must also allow for a reasonably accurate prediction of the total germination, obtainable from the seed batch, under nursery conditions.

Previous experience with germination behaviour and knowledge of conditions pertaining to the laboratory bench suggested that temperature was the major factor influencing seasonal variation in results. An incubator was obtained and attempts were made to carry out monthly tests at a standard temperature of 25°C. This temperature has been used to accelerate germination of *Pinus radiata* and also was the lowest temperature that could be consistenly maintained in the incubator, throughout the year.

The trials were a failure. Little or no germination could be obtained at 25°C, mould often formed on the seed under incubated conditions and it became obvious that no simple solution to the testing problem was available. As a result, suitable germination cabinets were ordered to allow for testing over a wide range of temperature and light conditions. Procedures to prevent fungal contamination were developed and a reproducible test procedure was evolved. Further trials were designed to fit the capability of testing equipment as it became available. Initially, the test programme was focussed onto the influence of temperature.

(i) Optimum Germination Temperatures

The failure of seed to germinate at 25°C and variability under ambient conditions posed the initial question as to what is the optimum temperature for germination. Monthly trials indicated that it should approximate the July-August conditions in Perth.

An initial shortage of controlled temperature cabinets necessitated that germination performance over a wide temperature range be tested in several trials. Results for a single large seed batch collected in South Lane Poole Block, Gnangara, are presented in Table 2.

TABLE 2

Percentage Germination Obtained Within Different Temperature Regimes. The Trials were Carried out in April and June with Non-stratified Seed Collected in South Lane Poole Block.

Germination Period	Germination Temperature			
	15°C	20°C	25°C	Ambient
14 days	27	10	1	19
28 days	75	48	4	58
Ratio (per cent)	36	21	25	33

Seed was unstratified and results are combined from two trials carried out in April and June. A temperature of 15°C proved most satisfactory and temperatures above this depressed germination. At 25°C the depression was severe and at 30°C zero germination has been consistently obtained. The latter result is not included in Table 2 as the separate trials at 30°C could not be tied in with ambient conditions defined in Table 2.

From the 14 and 28 day ratios in Table 2 it appears that temperatures which favour the highest germination percentage may also favour higher germinative energy.

Results expressed in Table 3 refer to a further trial testing the suitability of the 15°C temperature for two seed batches under both stratified and unstratified conditions.

TABLE 3

Percentage Germination with Temperature, Seed Batch and Stratification.

Germination period	Stratified			Not stratified		
	15°C	20°C	Ambient	15°C	20°C	Ambient
Seed S.L.P. 12/66						
14 day	68	45	52	26	10	18
28 day	89	75	86	78	69	48
Energy	76	60	61	33	15	37
Seed West Gironde						
14 day	62	41	52	19	5	12
28 day	90	73	92	72	37	73
Energy	69	56	57	26	14	16

Variation with seed batch is evident but for both lots the 15°C temperature provided maximum germination for both stratified and non-stratified seed. With stratification the germination was greatly increased but no great interaction was observed between stratification and temperature. Germinative energy was favoured by stratification and, to a certain extent, by the optimum temperature.

(ii) Seed Lots

To finalise temperature comparisons, use was made of four incubators to test seed concurrently at 10°C, 15°C, 20°C and 25°C. Seven different seed lots were employed to obtain a general result for a wide range of seed condition. These lots consisted of both stratified and non-stratified seed from the South Lane Poole and West Gironde seed production areas (G_1 E_1 S_0; G_1 E_1 S_1; G_2 E_1 S_0; G_2 E_1 S_1) and stratified seed from both sources which had been extracted without light exposure (G_1 E_0 S_1; G_2 E_0 S_1). The final batch was unstratified seed imported direct from Portugal (G_3 E_1 S_0). Inclusion of the three different seed sources and variation in stratification and light saturation was anticipated to cover as wide a range of germination possibilities as could be met in practice.

Two replications of 100 seed units were employed for each of the 28 treatment combinations. Table 4 includes the 28 day results for each treatment combinations.

TABLE 4

Percentage Germination Obtained for Seven Seed Lots under Four Fixed Temperature Regimes.

Seed Source	Germination Temperature			
	10°C	15°C	20°C	25°C
1. Imported Not Stratified (G_3 S_0)	3	37	25	13
2. S.L.P. Light. Not Stratified (G_1 E_1 S_0)	5	61	52	13
3. W.G. Light. Not Stratified (G_2 E_1 S_0)	2	62	52	15
4. S.L.P. Light. Stratified (G_1 E_1 S_1)	12	70	64	19
5 W.G. Light. Stratified (G_2 E_1 S_1)	8	65	73	24
6. S.L.P. No Light. Stratified (G_1 E_0 S_1)	11	66	65	17
7. W.G. No Light. Stratified (G_2 E_0 S_1)	6	48	60	26
Mean	6.4	57.0	56.6	18.1

Of the seven different seed batch combinations tested, five provided the highest germination at 15°C and two were highest at 20°C. Mean values at the four temperatures for all batches were 6.4, 57.0, 56.6 and 18.1 per cent. for 10°C, 15°C, 20°C and 25°C respectively.

The two greatest discrepancies from an optimum at 15°C are associated with the West Gironde seed batch (sources 5 and 7). The batch was a large one collected from a heavily thinned stand of 12 year old trees. It was collected at the same time as source G_1 from South Lane Poole which is a heavily thinned stand of 26 years of age. The original seed for both stands was imported from Portugal in different shipments. All seed handling from time of collection was identical for the two batches.

It will be noted in Table 3 that an earlier collection from the West Gironde seed production area gave significantly better germination at 15°C than at 20°C in a previous trial.

Results in Table 4 do not however, vary with seed origin alone. Results for treatments 3 and 5 (G_2 E_1 S_0 and G_2 E_1 S_1) indicate that stratification has altered the germination response to temperature in the West Gironde seed. Results for treatments 4 and 6 (G_1 E_1 S_1 and G_1 E_0 S_1) suggest that exposure of seed to light during extraction may also influence the response of germination to temperature. These data reveal that temperature is not the only factor to be considered in a standard testing programme and that a suitable standard temperature must be the one which favours most practical seed conditions and not necessarily all seed conditions.

Data provided in Tables 3 and 4 indicate conclusively that a temperature range of 15° to 20°C is desirable for a standard test procedure for *P. pinaster* in Western Australia. Consideration of the individual responses of the seven seed lots in Table 4 and those in Table 3, representative of a wide range of batch and handling conditions, indicates that the most favourable and consistent reponse was obtained at 15°C. This is considered to be the most suitable single temperature for standard test purposes.

(iii) Maximum Germination

Apart from testing fixed temperatures during germination, trials were made with fluctuating day and night temperatures of 20°C day and 5°C night and 20°C day and 10°C night. Fluctuating temperatures are more realistic and have often proved most beneficial in studies with other conifer seed. The temperature combinations tested were an attempt to approximate the normal ambient conditions in August when germination on the bench or, in the open, reaches a maximum value.

The fluctuating temperature regimes were either not as good as the 15°C fixed temperature or no better. Details of the trials are not reported as they offer no advantage over the material already considered.

The final consideration of temperature effects in seed testing techniques must be the precision in testing under the standard temperature conditions and the relation that test results have to the maximum under ambient conditions, i.e. whether testing at 15°C in a cabinet provides consistently reliable values which are equivalent to the values expected in the nursery.

Germination at 15°C was compared with germination on the bench for a number of monthly periods. Results are expressed in Table 5.

TABLE 5

Comparison of Percentage Germination at 15°C with Ambient Conditions

Month of Testing	Stratified				Non-Stratified			
	15°C		Ambient		15°C		Ambient	
	14 day	28 day	14 day	28 day	14 day	28 day	14 day	28 day
Seed S.L.P. 12/66								
April	....	....	....	....	28	71	19	47
June	68	89	52	86	26	78	18	69
July	....	....	....	....	29	82	13	59
September	79	91	80	96	37	82	39	85
October	....	....	....	....	27	82	31	79
Seed West Gironde								
April	....	....	....	....	20	59	5	34
June	62	90	52	92	19	72	12	73
July	85	95	65	87	....	....	....	....
September	83	97	79	92	38	82	19	73
October	....	....	....	....	16	72	20	73

Results for both stratified and non-stratified seed at 15°C in Table 5 are reasonably uniform within each seed batch and treatment. They are equivalent to the maximum germination obtained on the bench. Results for April at 15°C are inexplicably low and must be related to a variation in cabinet technique. This point will be discussed in the report concerning light requirements. Results from June to October in the cabinet were carried out with a constant light exposure during germination.

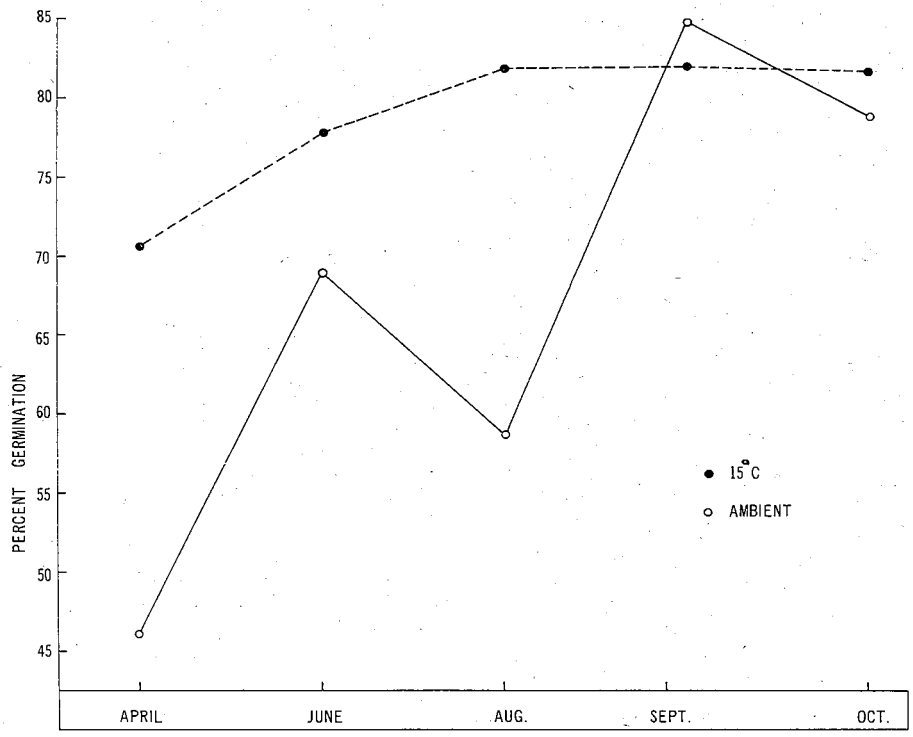

Figure 4.
Variation of germination percentages with season from tests within a cabinet at 15°C and on the bench under ambient conditions. Results for April in the cabinet were obtained with no control of light.

Figure 4 plots the results obtained for South Lane Poole, unstratified seed, in the cabinet, against results obtained each month on the bench. These data were analysed to show a highly significant month by temperature interaction. Except for the April value however, the results in the cabinet do not vary significantly with month.

Discussion

The complexity of the nature of germination in seed of *P. pinaster* is illustrated by the foregoing experiments. Seed source, year of collection, stratification and possibly light have complicated a simple definition of the most suitable temperature on which to base standard test procedures.

In commencing the temperature study, it was assumed that this factor had the greatest single influence on germination under standardised procedure. This has generally proved so and a range of 15 to 20°C can be definitely delineated as the most acceptable for practice. Most evidence supports the use of a temperature of 15°C for a standard testing medium. It has been suggested that variable performances, at this temperature, may be controlled by standardising other aspects of the testing procedure, i.e. stratification and light. These aspects are to be considered further in the report.

Repeated results with two large seed batches which are representative of local seed stand production (Table 5) indicate that testing at 15°C for at least part of the year can provide reasonable precision and accurate estimates of the maximum germination obtainable under ambient conditions.

The introduction of variable factors such as stratification and light and seed batches into this treatment of temperature response was unavoidable. It was essential to test a wide range of seed conditions to devise a useful standard testing technique. If a broad spectrum of seed had not been employed and a single batch had been tested, results obtained may have been misleading in general application.

In particular the response of seed from the West Gironde production area to 15° and 20°C temperature, for different years of collection, is emphasized (Tables 3 and 4). All trials were carefully implemented and replication within treatments provided minimum variation. Results were statistically analysed and there can be no doubt that treatment effects are real. This will become obvious when further factors influencing germination have been discussed in Section V.

Conclusion

For standard testing of seed of *Pinus pinaster* in Western Australia a temperature of 15°C is recommended. Temperatures below 15°C and above 20°C may drastically restrict germination.

III. THE INFLUENCE OF STRATIFICATION

Introduction

Hopkins (1960) has shown that stratification is beneficial to germination for the seed of the Portuguese provenance of *Pinus pinaster*. This determination supports previous knowledge concerning germination stimulation in the Landes provenance of the species (David and Guerindon, 1951) although stratification procedures varied somewhat in treatment times for the two provenances. An eight day soaking followed by seven to nine weeks stratification was found optimum for the Portuguese seed; longer stratification times proved less efficient. Both the cold soak and the stratification process were important.

From the initial work (Hopkins, 1960) it was recommended that further trials with a number of seed batches were desirable to separate a general treatment period suitable for routine seed. This report concerns further work carried out, since 1960, to define a general stratification procedure for operational use and standard testing procedures.

(i) Stratification Period

In the period 1958 to 1963 stratification trials were conducted annually with the seed of *P. pinaster* used for nursery sowing. All trials produced beneficial results from stratification, the improvement obtained being of a magnitude sufficient to recommend the procedure as a standard nursery prescription. The following points arising from these trials required recognition:—

(1) An eight day pre-soaking period proved superior to shorter periods.

(2) Soaking alone may have beneficial effects on the early results of germination.

(3) Soaking plus three to six weeks stratification at 3°C appeared to be optimum for the range of seed batches tested.

(4) Periods of cold storage of longer than nine weeks duration may depress germination below the maximum obtainable with shorter storage periods.

(5) Storage in polythene bags during the cold period is more satisfactory than packing in calico bags and a moist medium.

(6) Drying the seed naturally, following stratification, is acceptable but drying by electrical means has, in some instances, resulted in a depression in germination percentage.

Treatment response has shown considerable variation with different seed lots. Combined with rather variable results with respect to length of cold storage, the overall data still leave some doubt as to the optimum treatment for routine working. A further trial was conducted in 1964 to clarify this aspect.

Three imported seed batches, serials 3056, 3181 and 2875 were employed. The batches provided germination percentages of 85.6, 31.2 and 36.6 respectively, following a nine week germination period in sand flats. The following treatments were compared for each seed batch:—

(1) Control.

(2) 8 days soak.

(3) 8 days soak + 2 weeks cold storage at 3.4°C.

(4) 8 days soak + 4 weeks cold storage at 3.4°C.

(5) 8 days soak + 6 weeks cold storage at 3.4°C.

(6) 8 days soak + 8 weeks cold storage at 3.4°C.

(7) 8 days soak + 10 weeks cold storage at 3.4°C.

Soaking and placement in cold storage were arranged to allow all treatments to be completed on the same date.

Germination tests were carried out in petri dishes on the laboratory bench. Six replications of 100 seed units were provided for each treatment and results were tabulated for 7, 14, 21 and 28 days after commencement. The tests were carried out within the period September 28 to October 26.

Results for total germination at 7, 14, 21 and 28 day periods were transformed and analysed. Replication differences were not significant except at the seven day count. Significance at the 0.01 level was found for seed and treatment effects and for the seed by treatment interaction at each period of assessment. Mean percentage values for each treatment are compared in Figure 5. Results for the 14 and 28 day periods are presented as Table 6.

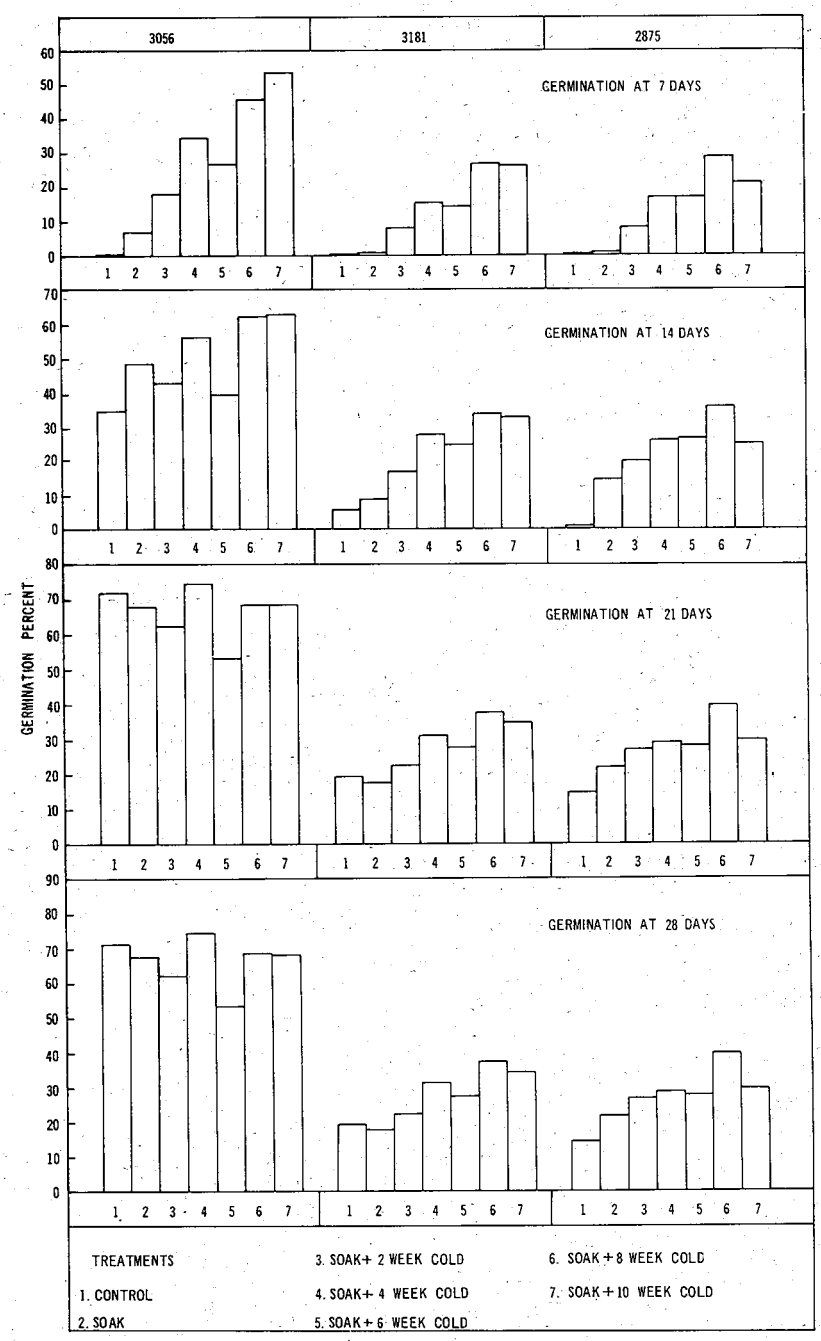

Figure 5.
Variation of germination obtained for three seed lots under seven soaking and stratification treatments.

With a seven days germination period the effects of treatment tended to increase with the length of cold storage (Figure 5). This effect is also apparent after 14 days when a depressing effect for treatment 7 (10 weeks cold storage) in serial 2875 is more pronounced and significantly different (0.01 level) to treatment 6. Treatment 5 for serial 3056 is also out of sequence and significantly less than treatments 4 and 6. This latter result must reflect an error in procedure. Up to 14 days, treatment 6 is definitely the superior

TABLE 6

Mean Germination Percentages for Stratification Treatments at the 14 and 28 Day Measurement Periods

Treatment No.	14 day Period				28 day Period			
	Seed lot				Seed lot			
	3056	3181	2875	Mean	3056	3181	2875	Mean
1.	34.2	5.5	0.9	9.8	80.4	26.2	20.1	41.8
2.	48.7	8.6	14.2	21.9	73.2	20.3	24.7	38.6
3.	42.9	16.9	19.3	25.6	69.7	25.3	28.2	40.6
4.	56.4	27.6	25.8	35.9	80.5	32.3	29.2	47.7
5.	39.6	24.2	26.4	29.8	60.9	28.4	27.8	38.6
6.	62.2	33.4	35.6	43.6	73.0	37.6	39.8	50.2
7.	62.8	32.7	24.4	39.4	70.6	34.4	29.5	44.8

At 28 days (Table 6 and Figure 5) no treatments in seed lot 3056 surpassed the control and treatments 3, 5 and 7 were significantly lower than the control. All treatments except 2 in serial 2875 were significantly greater than the control and in serial 3181 treatments 4, 6 and 7 were significantly better than the control.

On considering the range of treatments investigated, treatment 4 (soak plus four weeks cold storage) appears best suited to all seed batches. This treatment provided highly significant increases over the control for the three serials up to 14 days and for two serials up to 28 days. For germination periods longer than seven days, no other treatment gave results that were consistently and significantly higher.

TABLE 7

Percentage of the Maximum Germination Achieved as Obtained With and Without Treatment

Treatment	Serial 3056				Serial 3181				Serial 2875			
	Daily Period of Germination											
	7	14	21	28	7	14	21	28	7	14	21	28
1. Control	0	43	90	100	0	15	50	69	0	3	38	50
4. Soak + 4 weeks	43	71	93	100	38	73	82	85	47	70	77	79

The improvement resulting from treatment can be readily appreciated from Table 7. Stratification has greatly accelerated the germination rate in all serials. For two serials the germination percentage was increased, over the total period, with treatment.

The trial clearly illustrates the advantage available from pre-treating seed. For poor seed, both the rate and total germination may be increased over any practical field germination period. With good seed (3056) the total germination may be similar for treated and non-treated seed after a month but the rate, or germination energy, is increased greatly by pre-treatment. This latter aspect is important when considering survival in the nursery, timing for post emergent nursery operations and uniformity of nursery stock. For the three seed lots tested, soaking plus four weeks cold storage gave over 70 per cent. of the possible germination in 14 days, exceeding the control value by approximately two, five, and 20 times for different seed batches (Table 7).

The significant depression in total germination obtained in some treatments with the good seed (serial 3056) can be explained. Some seed had commenced to germinate on removing treatments from the refrigerator. On drying, this was killed and only a part of the total germination could be obtained with testing. This effect has consistently been recorded with good seed and is a problem which could be met in operations. It can be avoided by using cold storage temperatures of 2°C, instead of the 3.4°C used in the trial, and limiting length of storage to four weeks maximum. Since most routine seed to be available in future will have only minor dormancy problems, the eight day soak plus four weeks cold storage at 2°C is the recommended treatment.

(ii) Drying Following Treatment

In all experiments reported in this bulletin stratified seed was surface dried prior to testing. Drying normally consisted of exposure in open dishes in the sunlight or four hours exposure at 30°C in a forced draft drying cabinet.

The object of drying is to permit dusting with minimum quantities of fungicide prior to setting up each germination trial. Under operational conditions, seed drying is required to permit application of the fungicide and to allow smooth tracking through the seed drill when sowing. When drying, the criterion has always been to surface dry the seed rather than reduce it to a definite moisture content. Sowing is within several days of drying and no depression of the stratification effect results for the condition mentioned. Drying at higher temperatures and for longer periods in an electrically operated cabinet has caused depression from the maximum germination in one or two instances. This is probably the result of heat induced dormancy.

The dusted, surface dried, stratified seed has been stored at a temperature of 4°C for two months with no depression in germination. Other conditions of drying and post treatment storage have not been tested as they have no place in operational practice. Air drying at low temperatures and sowing as soon after treatment as possible is recommended practice.

(iii) Seed Source and Temperature

Significant interactions between seed source and the degree of response to stratification occurred in the previous trials. The fact that this may be a real effect is verified in a further trial.

Seed sources G_1 and G_2 were compared stratified (S_1) and unstratified (S_0) for Germination temperatures of 10°C, 15°C, 20°C and 25°C. A highly significant interaction between stratification seed source and temperature was recorded. It can be seen in Figure 6 that stratification has greatly improved the germination percentage for the one batch of seed at 20°C and 25°C. This effect is pronounced with the G_2 seed source.

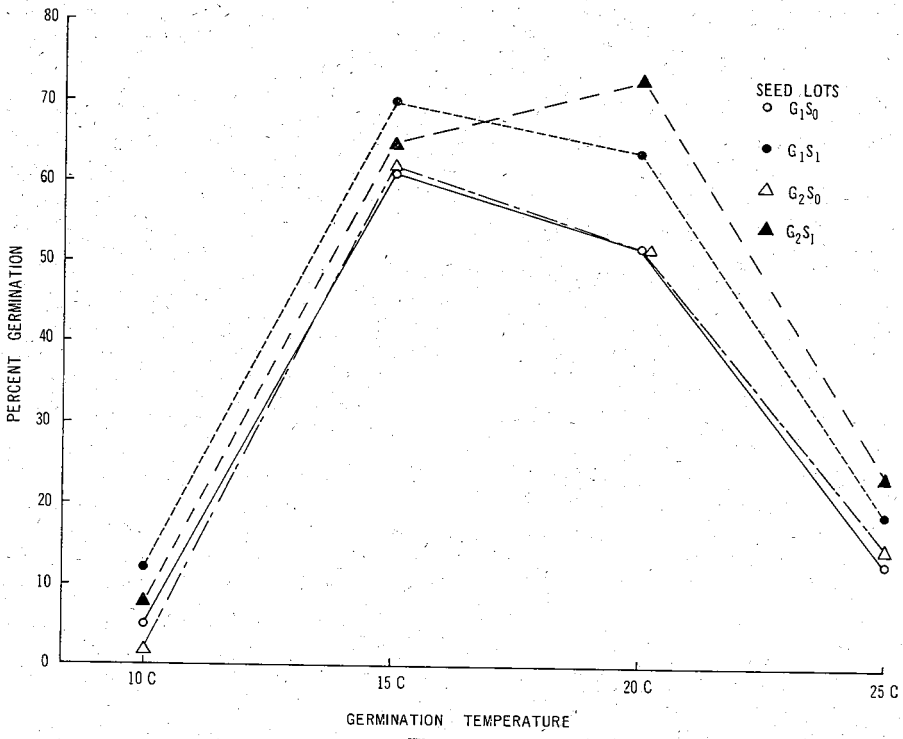

Figure 6.
The influence of stratification on germination at limiting and non-limiting germination temperatures.

Discussion

From Figure 6 it can be seen that stratification improves seed performance over the whole range of effective germination temperatures from 10°C to 25°C. The use of stratification as an operational procedure can be expected to be beneficial under any ambient germination condition.

The seed source by stratification interaction (variation in efficiency of response with seed lots) is common. It was shown in the first experiment (i) that this effect produces no complications in routine practice so long as unduly long cold exposure periods are not used.

Studies carried out were not designed to identify the physiological effects of stratification on *P. pinaster* seed. It has been shown however, that straight soaking can be beneficial to early germination responses. Also seed can be soaked for at least up to 14 days without any serious depression on viability. It would appear therefore that part of the stratification function is to assist imbibition through a reasonably thick and impermeable seed coat.

Stratification provides for rapid germination and most efficient germination over a wide range of temperature conditions. It will also be seen from Chapter IV that the stratification stimulus may operate independently to a light stimulus.

Many of the past problems met with in Western Australian nursery practice concerning low germination appear to be connected with poor storage conditions. Seed collected locally and recent importations from Portugal provide germination precentages in excess of 80 per cent. Full-sib seed produced in the breeding programme, on removing the empty or non-viable seeds which float on water, give germination percentages of 95 to 100 with stratification. Since past storage has been at room temperature, for periods up to four years, and no attempt has been made to control moisture content during storage, decline in viability and dormancy can be expected. Seed imported from Portugal has been tested to record over 10 per cent. moisture while in storage. Facilities have now been provided to store seed at 2 to 3°C with moisture content adjusted to below eight per cent.

Conclusions

Stratification is beneficial to germination in *Pinus pinaster* seed. A treatment comprising eight days cold soaking followed by four weeks stratification at 3°C is recommended for routine practice.

It has been shown experimentally that stratification may interact significantly with seed lots and temperature.

IV. THE INFLUENCE OF LIGHT

Introduction

Investigations to determine conditions optimum for germination testing of *Pinus pinaster* seed have revealed that both stratification following cold soaking and a constant temperature of 15°C are required. A further factor, light, was considered worthy of investigation.

In practice, *P. pinaster* is covered following sowing and light during the germination period is not considered an operative feature. In testing, however, variable cabinet design and different handling techniques normally expose the germinating seed to light at some stage of the investigation. The reason for the present phase of the study programme was to determine favourable light conditions which allow uniform and precise testing procedures under artificial conditions.

There is no previous published report concerning the positive effects of light on *P. pinaster* germination. From the general literature it is obvious that light of varying wave length and periods of exposure can influence the

germinative performance of many seeds. Despite the fact that no factual evidence existed to show that this was the cast with *P. pinaster,* some light influence was suspected. Although much of the variability associated with seasonal germination of *P. pinaster* can be removed by stratification and the use of favourable temperatures, minor inexplicable discrepancies in testing still have occurred from time to time (i.e., results for April in Table 5). It was believed these were related to a light effect.

For standard testing, a cabinet temperature of 15°C and 14 hours fluorescent light per day has been adopted. This lighting provided a constant standard condition which was convenient for testing with the facilities available and which certainly does not prevent satisfactory test results. With the possibility of testing under other cabinet conditions it is essential to determine whether alteration in the light conditions would significantly influence test results.

Investigations into light effects were carried out concurrently with temperature and stratification investigations. The report on this work is hence not separate from the previous two reports provided but is an adjunct to them. All studies were factorials incorporating temperature, stratification, light and often seed source. This section will emphasize and attempt to define the influence of the light factor.

(i) Preliminary Investigations

Prior to obtaining a suitable controlled incubator, several trials carried out on the bench provided for complete exposure to window light during germination (the normal practice) and complete exclusion of light. Although it appeared that light during germination had some beneficial influence, results within tests were extremely variable and inconclusive. These inconsistencies were considered to be associated with the variable ambient conditions on the bench and, possibly, with differences in seed batches. To define the situation it was decided to commence from the beginning, to use seed which had never been exposed to light and to work within a factorial framework.

(ii) Exposure Trials

In September 1966, a large batch of cones was collected from the South Lane Poole seed production area. Seed was extracted in an electrically operated kiln with half the cones in opaque bags to prevent exposure to light. The seed from these cones was extracted and stored in the dark (E_0). The other half of the cones was extracted, cleaned and stored with the normal exposure to window light (E_1).

A trial was designed to compare the E_0 and E_1 seed with stratification (S_1), without stratification (S_0), with light during germination (L_1) and without light during germination (L_0). Three replications of 100 seed units were employed and treatments were compared on the bench (T_0) in December and simultaneously in the cabinet (T_1) with 20°C day and 10°C night temperature.

On the bench, light (L_1) was normal window light. In the cabinet fluorescent lighting was provided during the 14 hour day period. A green safe light was used in setting up and counting the units, as necessary.

Treatment levels compared were:—

Temperature—

T_0—ambient conditions on the bench.

T_1—20°C day (14 hour) and 10°C night.

Exposure—

E_0—seed extracted and stored in dark.

E_1—seed extracted with window light.

Stratification—

S_0—not stratified.

S_1—stratified.

Light—

L_0—no light during germination.

L_1—14 hours light per day during germination.

Preliminary analysis of the 14 and 28 day germination results showed a significant third order interaction. This can be partly explained by the different light conditions used on the bench and in the cabinet. The trial was therefore evaluated as two factorials (Table 8), one under bench conditions (T_0) and one under controlled cabinet conditions (T_1).

TABLE 8

Analyses for Trials Comparing Pre-Exposure (E) Stratification (S) and Light (L) During Germination on the Bench and in the Cabinet

Source of Variation	Degrees of Freedom	Significance			
		Cabinet		Bench	
		14 Day	28 Day	14 Day	28 Day
E	1	.01	.01	.01	.01
S	1	.01	.01	.01	.01
L	1	.01	.01	.01	.01
E x S	1	.01	.01	.01	.01
E x L	1	.01	.01	.01	.01
S x L	1	N.S.	N.S.	.05	N.S.
E x S x L	1	.05	N.S.	.01	.01
Residual	14				
Blocks	2	N.S.	N.S.	N.S.	N.S.

Generally, germination under cabinet temperatures was slightly superior to that on the bench. The greatest portion of the treatment variation was accounted for by exposure (SS = 1550) with stratification (SS = 317) of lower importance. For both the cabinet and the bench trials the highest germination was obtained by the E_1 S_1 L_1 and E_1 S_1 L_0 treatments which varied only within a range of 69 to 74 per cent. The lowest germinations were obtained in the E_0 S_0 L_0 and E_0 S_1 L_0 treatments in both cases. These values were approximately seven per cent.

Both the E × L and E × S interactions were highly significant; the L × S interaction was not significant. The nature of these interactions is displayed in Figure 7.

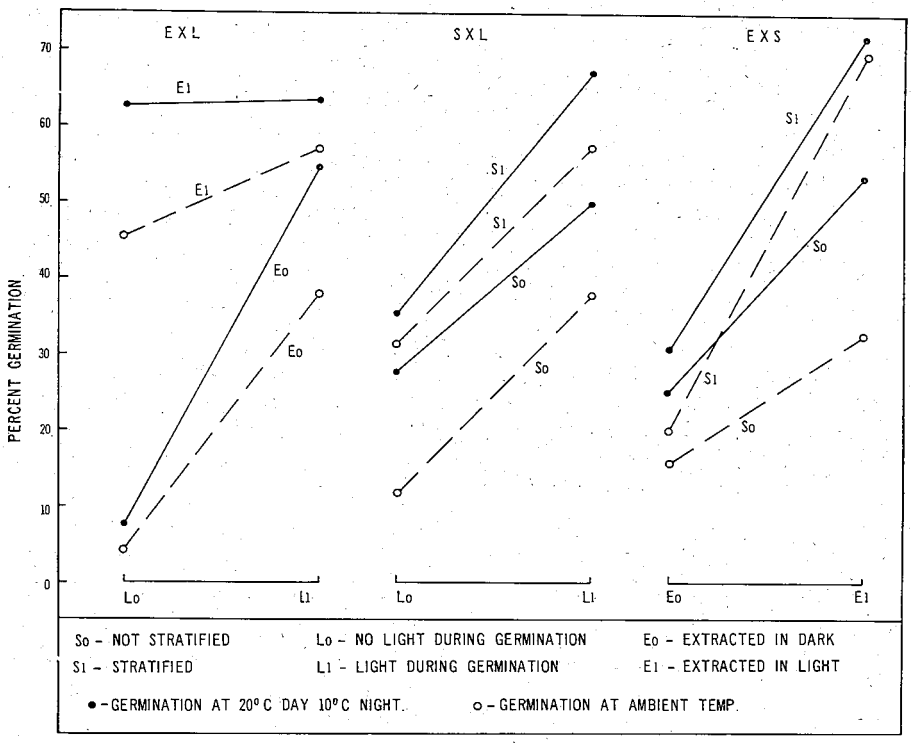

Figure 7.
Variation in germination with levels of stratification, germination temperature, pre-exposure of seed and light during germination.

Within the E × L interaction, the major influence is due to exposure. Under both bench and cabinet conditions seed exposed during extraction but without light during germination (E_1 L_0) provided superior germinations to unexposed seed subject to light (E_0L_1). In the cabinet a similar high germination value was obtained by the exposed seed with or without light. On the bench, where it is known that temperature was limiting, light during germination did improve the performance of the exposed seed. For unexposed seed without light (E_0 L_0) germination was almost a complete failure and light during germination, whether artificial or window light, greatly improved the germination.

The effect of exposure during seed extraction is dramatic and quite unexpected. Under both the cabinet and bench conditions, light exposure during extraction (several hours window light at the most) provided a superior stimulus to the almost constant light during the 28 day germination period. *P. pinaster* seed normally used on an operational scale is extracted in sunlight and would receive adequate pregermination exposure

The E × S interaction in Figure 7 shows that stratification improves germination of unexposed seed to some extent but has a much greater improvement with exposed seed. It would appear that stratification and pre-exposure of seed have separate and independent influences on stimulating

germination. The response was greatest with seed tested under limiting temperature conditions on the bench and a further influence of E × S × T is probable.

From the S × L interaction expressed in Figure 7, it is obvious that light during germination significantly increased germination for both stratified and non-stratified seed.

These results, provided with adequate replication and duplication under near optimum and limiting temperature conditions, leave little doubt of the importance of light to germination in P. *pinaster*. Fluorescent light and window light provided similar effects but were not as effective as light exposure during seed extraction. For exposed seed the pre-exposure completely replaced any further effect of light during germination, at near optimum germination temperature conditions. The importance of ensuring that seed has adequate exposure during extraction is obvious.

Inconsistencies of previous trials are acceptable with knowledge provided by the present study. Most seed tested previously had been extracted in sunlight and pre-exposure should have been adequate. Variability in trial results could have arisen with differences in stratification, light and temperature conditions.

It was considered essential to confirm the exposure effects for a range of different seed batches. This required seed extracted without exposure to light and could not be accommodated until a new cone crop was ripe in the following spring. In the interim period, two additional trials were conducted with the 1966 seed to further investigate effects of stratification and temperature.

(iii) Light During Stratification

Prior to stratification, seed is cold soaked for seven to 10 days. It has been reported that brief light exposures during stratification or while the seed is in an imbibed condition may materially influence germination response (Nyman, 1963; Ackerman and Farrar, 1965). A trial was carried out to determine whether light exposure during soaking or stratification, and different stratification periods, could produce variation in standard testing procedures.

Both exposed (E_1) and unexposed seed (E_0) was stratified for three periods of two weeks (S_1) four weeks (S_2) and six weeks (S_3) respectively. During the first week of the stratification, seed was exposed to both window and incandescent light for zero time (P_0), two hours (P_1) and 10 hours (P_2). The 18 treatments were germinated with light in the cabinet at 20°C day and 10°C night temperatures for four weeks. Three replicates of 100 seed units were employed.

No interaction proved significant after a 28 day germination period. Means for main effects were as follows:—

E_0 = 60.9	S_1 = 63.0	P_0 = 65.9
E_1 = 69.8	S_2 = 67.4	P_1 = 65.4
	S_3 = 65.7	P_2 = 64.6

Only the exposure main effect (E) proved to be significant. Neither light exposure during stratification nor length of stratification period had any real affect on decreasing the affect of lack of exposure during extraction. This statement is made with the reservation that some stratification was proved beneficial in the previous trial. The current trial purely shows that a two week period of stratification, with light during the germination period, is adequate to provide stratification requirements. Further, light during stratification in

this trial was only supplementary to some window light received in the cabinet during germination. It had no benefit over the brief exposure to window light during extraction.

(iv) Temperature

The unexposed (E_0) and exposed (E_1) seed was compared stratified (S_1) and unstratified (S_0) under the three temperature conditions of 15°C (T_1), ambient in February (T_2) and 25°C (T_3). Germination was evaluated only over a 14 day period.

The interaction between exposure and stratification was significant, the stratification being more effective on exposed seed, as in Figure 7 for trial (ii). Mean germination percentages for main effects were as follows:—

E_0 = 8.3	S_0 = 11.3	T_1 (15°C) = 35.6
E_1 = 28.8	S_1 = 25.8	T_2 (ambient) = 10.4
		T_3 (25°C) = 9.7

Very real effects of exposure, stratification and temperature were recorded. It should be noted that the 28 day result for the E × S interaction in the previous trial (iii) was just not significant. At 14 days it was highly significant, as with the present result.

A mean germination of 71 per cent. was obtained in the E_1 S_1 T_1 treatment at 14 days. This was comparable with the best result obtained in the first exposure trial (ii) for the same germination period. The current trial confirms results in the previous trials while showing that the same limiting temperature conditions apply to both exposed and unexposed seed.

(v) Different Seed Batches

In September 1967 two separate seed batches, one from the South Lane Poole seed stand (G_1) and one from the West Gironde stand (G_2) were collected, extracted and stored as pre-exposed (E_1) and unexposed (E_0) lots. Germination was compared in the cabinet at 15°C for stratified (S_1) and unstratified (S_0) conditions with no light (L_0) and 14 hours fluorescent light per day (L_1). Three replications of 100 seed units were employed for each of the 16 treatments compared. When necessary, the (E_0) and (L_0) treatments were manipulated and counted under a green safe light.

Results for analyses of the 28 day germination counts are set out in Table 9.

Mean percentage germination at 28 days for the experimental main effects were as follows:—

G_1 = 35.9	E_0 = 25.1	S_0 = 32.1	L_0 = 20.6
G_2 = 39.1	E_1 = 49.8	S_1 = 43.2	L_1 = 54.5

For germinative energy, the E × S interaction was significant. Differences between seed batches were not found to be significant in analyses.

Results for the E × S × L interaction for both the 14 day and 28 day counts are shown in Figure 8. At 14 days the highly significant effect of stratification on E_1 seed is paramount. At 28 days, results are quite regular showing definite advantage for stratification, light and exposure.

Results for individual seed batches in the 1967 trial and the 1966 South Lane Poole seed treated in the cabinet (G_3) are compared in Figure 9. The very real effect of pre-exposure (E_1) on germination is evident for all three

batches. Maximum germination was obtained in all cases with pre-exposure, stratification and light during germination. Without pre-exposure or light, germination was a virtual failure even with stratification. Stratification is obviously not a replacement stimulus for the light effect but exposure and light can provide a major stimulus in the absence of stratification.

TABLE 9

Analyses of Results for the 1967 Seed Exposure Studies

Source of Variation		Degrees of Freedom	Significance	
			28 Day Germination	Germination Energy
Source	G	1	N.S.	N.S.
Exposure	E	1	.01	.01
Stratification	S	1	.01	.01
Light	L	1	.01	.05
G x S	....	1	N.S.	N.S.
G x E	....	1	N.S.	N.S.
G x L	....	1	N.S.	N.S.
S x E	....	1	N.S.	.01
S x L	....	1	N.S.	N.S.
E x L	....	1	.01	N.S.
G x S x E	....	1	N.S.	N.S.
G x S x L	....	1	N.S.	N.S.
G x E x L	....	1	N.S.	N.S.
S x E x L	....	1	N.S.	N.S.
Residual		30		
Blocks		2	N.S.	N.S.

The value of Figure 9, apart from confirming general results in both trials, is to observe the variable effect of seed source on results. Seed sources 1 and 3 were from the same seed stand collected in different years. A major difference in results between these two lots is for the exposed (E_1) seed. In 1966 it appeared that pre-exposure fully provided the light requirement under cabinet conditions but not on the bench (Figure 7). The same seed source in the 1967 collection, and the West Gironde seed source, reveal however, that pre-exposure may not necessarily provide total light requirements. Light during germination was as effective as pre-exposure.

Differences in response with year from the South Lane Poole seed could have been due to different pre-exposures or environmental by source interactions. The following report on variation in germination with seed maturity would suggest that the latter possibility is the pertinent one. Pre-exposure in 1967 and 1966 was identical from all experimental considerations.

Discussion

These trials demonstrate without doubt that light is a factor of major importance to *P. pinaster* germination. Light may influence germination results whether exposure is during seed extraction and cleaning or during the actual germination process. The E × L interaction obtained in exposure

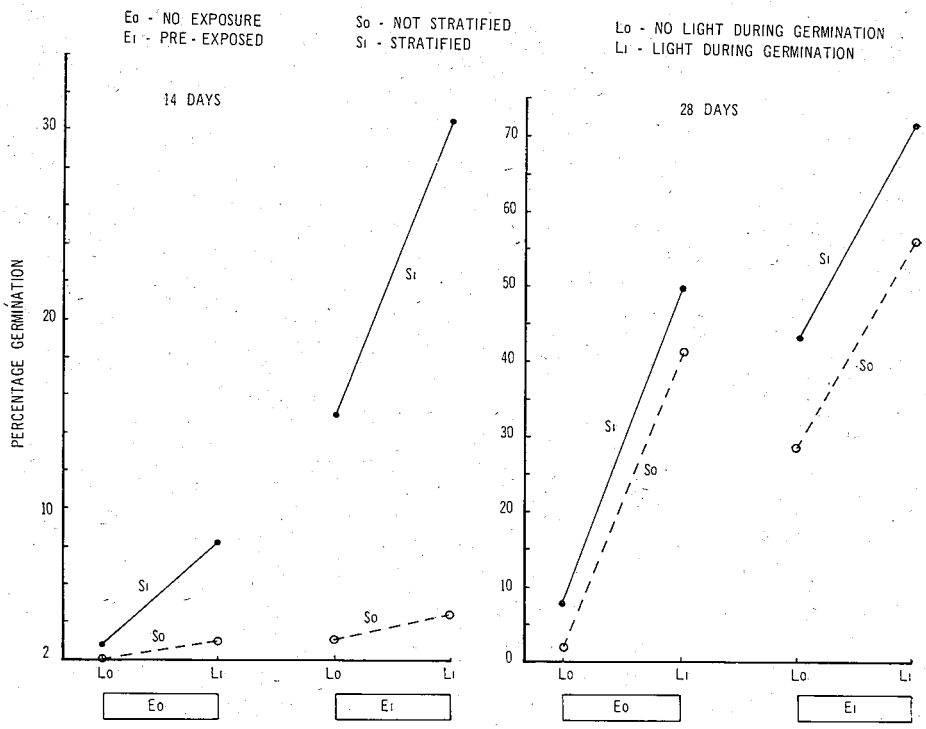

Figure 8.
Influences of pre-exposure, light during germination and stratification obtained in the second exposure trial. Results are means for two separate seed batches tested.

trials (ii) and (v) were highly significant and incorporated different seed lots and different temperature regimes. This interaction for trial (v) is of similar trend to that displayed for germination at ambient temperature in Figure 7. Pre-exposure and light were not consistently equivalent in effect nor was either means of irradiation consistently associated with the total response to irradiation obtained. Perhaps the most significant result of the whole study is the effect that a brief exposure of indirect window light, during seed drying and extraction from the cone, has on the overall behaviour of germination.

In normal operations, seed is extracted from the cones by exposing them to the sun for several days. This degree of exposure is infinitely greater than that provided in the trial. Several pilot trials comparing light during germination with no light, for such seed, produced conflicting results for a light effect. It is possible that exposure during natural seed extraction may fully satisfy the light requirement of the species.

Stratification does not replace the light effect but may interact to produce a greater response with light treatments. This effect is not great after 28 days' germination and, for light saturated seed, may not be a significant effect. Studies of stratification which have produced significant anomalies under different germination temperatures and with different seed batches

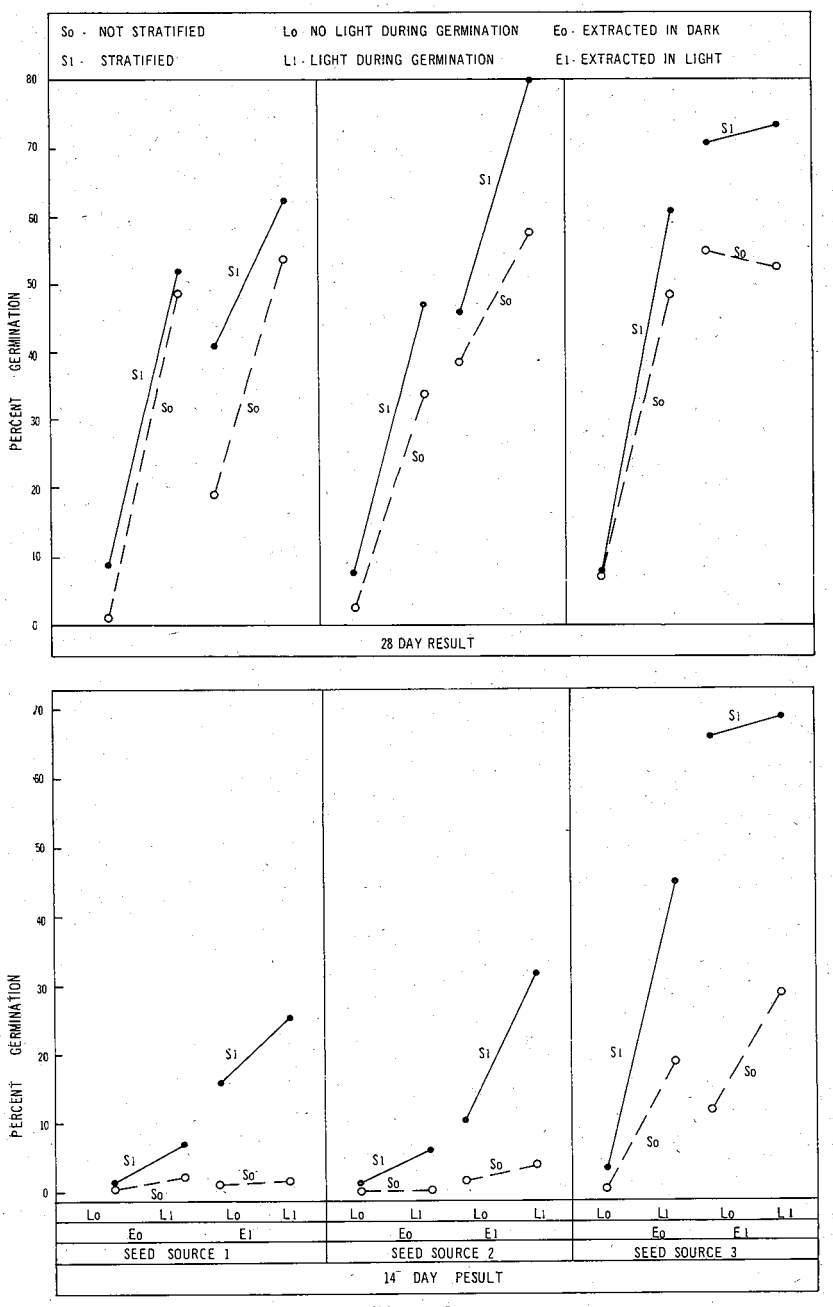

Figure 9.

Comparison of results obtained in the exposure trials for each of the three seed lots employed. Seed sources 1 and 3 are from the same stand collected in different years. Variable results reflect variable test conditions and environmental factors.

probably reflect the exposure by stratification by temperature interactions obtained in current trials.

This work by no means explains the mechanism of stimuli by light and stratification on seed germination. It does in fact open up a most interesting field for basic study. Trials have been adequate to show that light is a major factor in the germination process of *P. pinaster* seed. For routine testing it is essential to see that seed receives adequate exposure before storage or testing. Probably this exposure should be by sunlight when the seed is at a high moisture content. It is also considered that light during routine testing is to be recommended. This exposure would not lower germination results and, in limiting circumstances, may increase them slightly and lead to precision in testing (Figure 4).

Conclusion

Light is a major factor which influences the germination of *P. pinaster* seed. Natural extraction and stratification probably ensure that light exposure is not limiting to germination in nursery beds. In standard testing techniques it is considered advisable to provide light during the germinative period.

V. THE INFLUENCE OF SEED MATURITY AND GENOTYPE

Introduction

Within the tree improvement programme in Western Australia it is general practice to collect hand-pollinated cones of *Pinus pinaster* in October and November and to extract the seed directly, following drying in the sun. Even in extremely hot, dry summers, cones do not naturally open and commence to shed seed from the tree before early December. Local collection practice is hence based on the premises that maximum ripening time under natural conditions is desirable. Results from such collections have given no reasons to doubt this premise and, combined with the relative ease of drying and extraction at the particular time of the year, cone collection in early summer is a satisfactory practice.

The practice of taking cones from the tree earlier than spring offers some operational advantages. Early collection from the orchard reduces the time of exposure to birds and hence could restrict cone losses by cockatoos *(Calyptorhynchus baudinii)*. The ability to use immature cones dropped to the ground by birds could markedly reduce collection costs in plus stands. Collection during the extended period March to October could also make better use of labour available for seed collection.

Early studies of cone maturity showed promise but were not replicated or sufficiently well-designed to serve as a basis for operations. In 1967 a further study was carried out to positively determine the earliest month in which cones could be satisfactorily removed from the tree for operational seed extraction. This study revealed that conditions for cone maturation markedly influenced germination results.

Procedure

All collections tested were made in 1967 from clones planted in 1959 and 1960 in the Neaves Road Scion Arboretum.

To allow for tree to tree variation, five cones from the grafted clones E19, E22, E32, E37 and 10 cones from E24 and E47 were collected each month from January to October. Each clone was represented by at least ten ramets and it was possible to pick large, healthy cones throughout the study.

Following collection, cones for each clone were retained separately in ventilated polythene bags in a cool, dark cupboard. Seed from all batches was extracted in November 1967.

Two germination trials were employed to test the seed.

Trial A. Unstratified seed of monthly collections from clones E19, E22 and E37 were tested in January 1968, under both ambient and 15°C temperatures.

A 100-seed unit without replication was tested under each temperature condition. Prior to testing, the seeds were soaked for eight days in water, all floaters were removed and the surface-dried seeds were dusted with "Captan". Germination counts were made at weekly intervals for four weeks and 14-day, 28-day and germinative energy data were analysed.

Trial B. The second trial compared collections in March, May, July and September for clones E24 and E47 under both stratified and unstratified conditions. Germination was at 15°C in a controlled temperature cabinet. Three replications of 100-seed units were employed for the 16 treatment combinations. Results for 14-day and 28-day germination and germinative energy were analysed.

Following extraction, variation in seed size, colour and weight between months and clones was obvious. Variation in seed weight and percentage floaters (empty seed) is shown in Table 10.

TABLE 10

Variation in Weight per 100 Seeds and Percentage of Empty Seeds within Clones for Different Months of Cone Collection

Month of Collection	Seed Origin								
	E24	E47	E32	E19		E22		E37	
	Wt (g)	Wt (g)	Wt (g)	Wt (g)	%	Wt (g)	%	Wt (g)	%
January	4.1	2.9	4.8	4.3	14	3.9	20	2.6	15
February	4.4	3.5	6.5	6.0	8	5.4	20	4.3	9
March	5.4	3.7	6.9	7.1	9	7.2	4	4.4	11
April	7.4	3.9	6.5	7.1	4	6.1	8	4.0	15
May	6.6	4.0	6.6	7.0	7	6.0	11	4.3	8
June	7.0	4.2	7.7	7.1	6	6.3	10	4.2	10
July	6.7	4.6	7.1	7.0	14	7.7	6	4.7	12
August	7.1	3.9	7.4	7.2	8	6.3	8	4.5	17
September	6.7	4.2	7.2	7.8	5	6.2	8	4.4	6
October	....	4.1	....	7.5	7	6.6	8	4.4	11

From this table it will be seen that the weights of 100 seeds for all clones were lowest in the January and February collections. Seed from most clones

had reached maximum size and weight by April. Generally, most seed collected after March appeared to be normal. January and February collections were light in colour, small in size and looked abnormal.

Percentage floaters obtained for clones E19, E22 and E37 in Table 10 vary greatly. This may be the result of non-uniform cleaning procedures as the small batches involved had to be hand treated. Winnowing could have varied in effectiveness with size of the batch or size of seed. Normally, good quality seed from large local collections weighs approximately 6.0 grams per 100 and contains about 4 per cent. of floaters. Such seed provides between 80 and 90 per cent. germination following stratification.

Time of Collection and Germination

Results for the 28-day germination period in Trial A are presented in Table 11.

TABLE 11

Percentage Germination of Three Clones for Seed Collected from the Trees in Different Months

Clone	Replication	Month of Collection									
		Jan	Feb	March	April	May	June	July	Aug	Sept	Oct
E19	A	0	3	40	29	36	34	0	36	2	5
	C	0	13	47	59	47	73	1	50	15	42
E22	A	0	0	73	32	84	86	79	75	64	72
	C	4	4	87	44	94	99	99	69	96	91
E37	A	0	0	11	17	19	20	26	10	3	4
	C	0	0	57	21	53	52	63	65	71	29
Mean		0.7	3.3	53	34	56	61	45	51	42	42

A—Germinated on the laboratory bench—Ambient.
C—Germinated at 15°C in a cabinet.

Variation in performance by the three clonal groups was considerable. Mean values for the 10 monthly collections for E19, E22 and E37 were 27, 63 and 26 per cent. respectively. Maximum values received for any one month were 59, 99 and 71 per cent. respectively. Since all ramets were adjacent, on a uniform site and of identical age, clonal differences displayed can be attributed to genetical causes. The pollen cloud available for fertilisation came from over 40 clones on the area and an adjacent plantation.

The monthly trend in germination was also not consistent for each clone. In all instances the germination from January and February collections was virtually a complete failure. For E19 and E22 the maximum germination was obtained for the June collection and for E37 it was July. These high values were not maintained for the remaining later collections as expected. In fact, E19 failed to germinate from the July collection and was greatly depressed in September, E22 was low in April and both E19 and E37 were severely

depressed in October. Although germination was consistently better in the cabinet at 15°C than under ambient conditions on the bench, the trends expressed above are reasonably consistent for both temperature conditions.

Stratification

Trial B was designed to confirm results in Trial A and to see whether stratification influenced the final germination values of seed from prematurely picked cones. Results are expressed in Table 12.

TABLE 12

Percentage Germination of Stratified and Non-Stratified Seed from Cones of Two Clones Collected at Four Stages of Maturity

Month of collection	E24		E47	
	Stratified	Not Stratified	Stratified	Not Stratified
March	32	15	96	96
May	84	73	80	77
July	47	42	41	42
September	95	93	99	96
Mean	56	19	74	64

From Table 12 it will be seen that stratification has not removed the variation associated with time of collection. For E47 near maximum generation was obtained in the March collection while the highest value for E24 was in September. It is of interest to further note that for both seed lots, whether stratified or not, the depressed germination for the July collection, evident for E19 in Table 11, was repeated for new data in Table 12. The influence of stratification also interacts significantly with month of collection and genotype.

Discussion

The excessive variation in seed performance, as expressed for the separate clones, has been experienced consistently in germination studies for *P. pinaster*. In this instance it can be associated with clonal characters. In previous studies similar variation has been associated with seed lots from different stands of the same age, stands of different ages and for collection from the same stand, in different years. It is obvious that germination performance is dependent upon both genetical and pre-conditioning factors which are not understood. Within this study, examples of the latter situation are the unexplained low germination for certain batches in July and to a lesser extent in April, September and October.

Size of cone, method of collection, storage procedure and extraction procedure were identical for all collections. Why E19, E24 and E47 should show depressed germination in July when E22 and E37 provided near maximum performance is inexplicable. Particularly as the clones with depressed germina-

tion gave near maximum values for collections in previous and following months. Apparently some environmental effect in June or July affected these clones and not the others.

Possibly the unexpected low germination values obtained for some clones in September and October also reflect a similar clonal-environmental interaction in this period. These unexplained values are of interest in that they show that seed set and ripening is a complex process and not as straight forward as one may wish to believe. With large sums of money involved in tree breeding and seed orchard establishment throughout the world it is considered that basic studies into factors associated with seed set and seed ripening would be well repaid.

For operational practice one must consider the trial results with respect to large batches of seed representing a broad, rather than a narrow, genetic basis. On this scale, the results from the two trials can be pooled and it is safe to say that cones picked from March onwards (19 months after fertilization) can provide satisfactory seed for plantation establishment. This recommendation assumes that such collection will be stored until spring in a satisfactory manner and extraction will take place in early summer.

Conclusion

Results of the study reveal considerable variation in germination performance due to clone by environmental interaction. This phenomena is unexplained in our present state of knowledge and warrants further study. For large scale collections, as normally apply to operational procedures, the genotype environmental interaction need not cause great concern at present.

In standard procedures to assess germination behaviour it is essential to ensure that the test sample is representative of the seed batch. Unrepresentative samples could lead to excessive variation in results or misleading results, or both.

VI. GERMINATION REQUIREMENTS

Introduction

The objective of the work programme reported was to provide a technique for testing germination in *Pinus pinaster* which will allow confidence and precision in nursery sowing. This objective has been achieved.

The recommended test procedure incorporates the following features:—

(1) Seed extraction to allow exposure to a light stimulus.

(2) Seed storage at 3°C in sealed containers with the seed moisture content adjusted to eight percent.

(3) Prior to germination the seed is soaked in water at room temperature or less for five to eight days.

(4) The water and floating seeds are drained from the viable seed which is then placed in sealed polythene bags for four weeks at 3°C.

(5) Stratified seed is surface dried, preferably in sunlight or in a forced draft at 30°C or less.

(6) For testing, the seed is lightly dusted with "Captan" fungicide and set out in 100 seed units on filter paper over an inverted watch glass (or on vermiculite) in a petri dish. The filter paper or vermiculite are kept moist by adding water as necessary during the incubation period.

(7) Germination potential is assessed by incubating the seed at 15°C with 14 hours lighting per day for 28 days. Provision of light is not essential but either standard light or no light system is desirable for maximum precision between tests.

The germination value obtained represents the maximum possible with stratified seed sown in the nursery in August. Variation in weather and other seasonal conditions will often produce less than maximum performance in the nursery. Provided spacing is regulated in the nursery rows according to the predicted germination, later germinations, delayed by inclement weather or deep sowing, should develop into plantable stock. This possibility is lessened if seed density within rows is increased above predicted values.

Current nursery practice developed for seed bed sterilization and forming, seed dusting and sowing, weed control, irrigation and fertilization should realize the predicted germination potential. This however, can only be ascertained from experience over several years of operations.

Any framing of operational prescriptions can be done only with the realization that improvement is always possible and variations will be warranted to meet specific circumstances. The complexity of germination behaviour found in this study and limitations in study procedure also reveal that knowledge of the subject is by no means complete. It is therefore considered essential that test procedures and nursery results should be continually referred to the findings of this study. At some stage operational results will again be found limiting and further development of the investigation will be warranted.

This final section of this report summarizes what is known and, in some instances, what isn't known concerning germination in *P. pinaster*.

Seed Source

The investigation was undertaken on the understanding that future seed would be obtained from seed orchards and local seed production stands. In most instances, the interactions associated with seed source in the study will be accounted for by testing and will not seriously influence operational results. They could lead to errors in testing and variability in the nursery unless seed batches are adequately subsampled for testing and mixed before sowing.

The variation in germination attributable to the effects of genotype and interaction between genotype and environment is also not necessarily important to nursery procedures provided seed orchards contain a number of genotypes and seed is well-mixed before using. It is probable however, that variable response to environmental preconditioning, detected in this study, represents an important aspect of crop yield. Improved knowledge of the subject may lead to economic advantages in managing seed orchards.

The variation in germination associated with seed source, determined for collections from different seed stands, or for the same seed stand from different years of collection, points to the desirability of replicating seed sources for experimental work on germination. There is no unique response for the species but rather a broad pattern of behaviour in germination which can be related to certain ranges of environmental stimuli. Germination techniques in the present programme provided excellent agreement between replicates. Two replicates were normally adequate and, particularly in large factorial experiments, replications of seed sources offered greater information and confidence in results than did replications within treatments.

Season of Germination.

Germination carried out each month of the year either in the open or on the laboratory bench showed great variation (Figs. 1 and 2). Sowing time was found to be critical. The most suitable time for nursery sowing in the Perth Metropolitan climate was determined to be August. In southern nurseries this season could be increased in range to late August or early September.

The use of stratification, constant temperature and light in a controlled incubator has provided consistent, high germination values over the period April to October (Fig. 4). Maximum germination values have also been obtained with different seed lots tested over the summer months.

David (1951) noted that a seasonal rhythm was experienced with French seed of *P. pinaster* germinated in the dark under constant conditions of temperature and humidity. This was considered to result from an inherent cyclic rhythm and not through environmental stimuli. It is desirable to determine whether such a condition actually exists with Portugese seed of a single batch tested monthly under the standard technique.

For operations, the possibility of variation in test results with season is no problem provided the nursery prediction is made from tests conducted in the period April to August.

Stratification

Numerous tests indicate that a five to eight day soak in water plus three to four weeks stratification at 3°C is adequate to provide optimum benefit with seed of Portuguese origin. Removal of floating seed while soaking can provide for germination of up to 95 per cent. with fresh seed. In practice the cold soak is carried out at 3°C in the refrigerated room. This is convenient and reduces the possibility of mould formation. It is not essential and all early work was soaked on the bench at room temperature. It should be advantageous to change the water several times during the soaking process and this is normally practiced.

In no aspect of this study has poor seed, i.e seed with a very low untreated germination value, been improved by stratification to the fresh standard, i.e. untreated seed with over 60 percent germination (Table 6). Germinative energy can be greatly improved and total germination can be significantly increased with poor seed. But if seed is allowed to become very dormant or allowed to deteriorate through faulty handling or storage procedures, stratification or any other known means will not rejuvenate it. Proper attention to collection, extraction and storage procedures must be provided to reduce the dormancy problem.

The study has shown that stratification and incubation temperature may interact to favour germination. The influence of adverse temperatures on germination is not effectively ameliorated by stratification and stratification has no useful effect in improving germination in temperatures outside the optimum range of 15 to 20°C (Fig. 6).

It has also been shown that stratification has no practical effect in improving seed with a low germination potential induced by environmental preconditioning (Table 12). Germination of unfavourable, preconditioned seed is improved by stratification but not restored to the maximum level.

Stratification has no important positive influence on germination if the seed has not received a light stimulus (Figs. 8 and 9). As with temperature, stratification will not ameliorate germination in seed with limiting light stimuli but it can positively interact to improve germination where light stimuli is near optimum.

From the practical advantage of improving germination energy in all operational seed (Table 7) stratification is warranted in *P. pinaster* nursery practice.

Temperature

Temperature is considered to be the major single factor influencing germination in *P. pinaster*. Sowing nurseries in August is critical to nursery results.

Adaptation to a narrow temperature range is highly developed in this species. The optimum temperature range, determined as 15° to 20°C, is low for commercial species. The tolerance range is also low and the severe depression or restriction in germinations obtained at temperatures of 25°C and higher explains some of the reasons for the great seasonal variability peculiar to this species (Fig. 1).

For standard test procedures, a fixed temperature of 15°C is most satisfactory and necessary for precision between tests. In several trials it was observed that an increase in temperature from 15°C to 20°C, for only three days, appeared to depress germination below the optimum. The sensitivity of the species to relatively minor temperature changes is considered to be a major finding from the study.

The influence of temperature in inducing dormancy in seed of this species would be an interesting study. It is known that the buds enter into a dormancy condition at the end of the summer and require cold treatment for relief (Perry and Hopkins, 1967). Depressed germination has often been associated with drying stratified seed in an electrically heated cabinet without forced ventilation and temperatures of 25°C and 30°C virtually prohibit germination.

It is probable that the effects of high temperatures on dormancy are only operative when seed is exposed while in an imbibed condition. Recently, imported seed was subjected to temperatures of 65°C for 0, 4, 8 and 24 hours to assess the influence of possible quarantine proposals. The seed was unstratified with a moisture content of approximately 10 per cent. Germinations obtained from the above treatments were 27, 31, 31 and 29 per cent. respectively, showing no significant variation. The seed was, however, of poor quality and does not necessarily reflect the response of fresh seed under similar treatment.

Present evidence suggests that heat sterilization of dry seed does not induce dormancy while, with imbibed seed, temperatures of 25° to 30°C virtually prohibit germination. For normal storage, temperatures of 3°C and seed moisture contents of eight per cent. or less are recommended.

Light

The study reveals that light stimulus is essential for germination in *Pinus pinaster*. Even a brief exposure to indirect window light during extraction may have a dramatic effect on the success of germination. The normal exposure to sunlight during extraction is apparently adequate to fully satisfy the light requirement of fresh seed. Whether this stimulus is maintained over long storage periods was not determined.

Pinus pinaster can be added to an appreciable list of pine species—*Pinus banksiana, P. caribaea, P. contorta, P. densiflora, P. echinata, P. mugo, P. nigra, P. rigida, P. palustris, P. ponderosa, P. strobus, P. taeda, P. thunbergii, P. virginiana, P. sylvestris*—quoted by Nyman (1963) as being susceptible to a light stimulus for seed germination. For *P. sylvestris,* Nyman (l.c) found that a brief irradiation was adequate to provide the necessary light stimulus for germination under dark conditions, and that imbibition was not essential for the seed to receive the irradiation stimulus. The stimulus was most effective when seed was irradiated several hours after imbibition commenced. The stimulated germination, following irradiation of the unimbibed seeds, decreased with storage but was still significant after 17 months' storage.

Ackerman and Farrar (1965) found that jack pine germination under continuous light was rapid and complete in seven to eight days at incubation temperatures of 21 to 27°C. Germination was also complete at 16°C but a longer incubation period was required. Germination was markedly reduced at all temperatures with the exclusion of light. Light did not become effective in controlling or "triggering" germination until a threshold moisture content of approximately 10-20 per cent. of dry weight was attained. Once this threshold moisture content was attained exposures of two to four minutes provided complete germination at all temperatures tested.

These more detailed studies, cited for Scots and jack pine, suggest further useful studies of the light effect on *P. pinaster* seed. To a large extent they support the limited studies carried out in the present programme and it is believed that current investigations are adequate to achieve the goals of the programme.

For standard test procedures it is essential to ensure the seed has received irradiation during extraction. To make sure of the pre-exposure when seed has been stored it is considered advisable to carry out the seven-day pre-stratification soak in the presence of window light. Light during the incubation period should further ensure that test results are optimal and maintain precision between tests. Pre-soaking seed for operational use in the presence of window light and surface drying in sunlight should also ensure that adequate light stimulus is operative.

Environmental Preconditioning

The study of genotype and seed maturity was initiated with the major objective of improving seed orchard management in the presence of bird

depredations, rather than to assist the seed-testing investigations. It has shown that cones taken from the trees in March, nineteen months after fertilization, may be used to produce seed for operational use.

Variable germination behaviour, depending on the period in which the immature seed was retained on the trees after March (Tables 11 and 12), explains why variation was found in other studies—with seed batches from a single production stand but collected in different years. Clonal variation, which can be related to genotype, was not unexpected and, with these clones, has previously been shown to influence graftability (Hopkins, 1966), nutrient uptake, the pattern of shoot elongation and other physiological and morphological reactions. The sensitivity of immature seed to environmental preconditioning and its great effect on germination performance was not fully appreciated.

There is nothing new in the concept of environmental preconditioning (Koller *et al*, 1962; Rowe, 1964). Possibly, the effect, its reversibility with time and the extent of the range of influence with stimulus (Tables 11 and 12) has not previously been so dramatically displayed for conifer seeds, as in this present study. With clone E47, for instance (Table 12), germination of seed collected in March, July and September varied from 96 to 41 to 99 per cent. respectively. It is believed that the order and nature of this variation in effective seed yield must warrant detailed study within the economics of seed orchard management.

Of immediate interest is whether preconditioning has altered the light or temperature requirements of the seed. Within the present study it was only possible to assess the influence of stratification and to show that this has little effect in reducing adverse influences caused by preconditioning. A whole new field of research with preconditioned seed lies open and its pursuit may greatly improve our knowledge of seed physiology.

Conclusions

The standard test devised for seed of *P. pinaster* in Western Australia adequately accounts for light, temperature and stratification requirements to provide optimum germination in any month of the year. Application of results from such tests to determine the number of seeds to sow and their espacement should materially improve local nursery practice.

LITERATURE CITED

Ackerman, R. F. and J. L. Farrar, 1965. The effect of light and temperature on the germination of jack pine and lodgepole pine seeds. Tech. Rep. Fac. for. Univ. Toronto No. 5 p. 41.

Bonilla, J. A. and C. A. Rava, 1963. Comparative study of physical, biochemical and physiological viability tests compared with the classical germination method for *Pinus pinaster*. Bol. Dep. For. Uruguay 3: 9-15.

Bonilla, J. A. and C. A. Rava, 1963. Hastening germination in seed of *Pinus pinaster*. Bol. Dep. For. Uruguay 5:1-6.

David, R., 1951. L'activation de la germination des graines de pin maritime. Bois et Resin. 32: 1551.

David, R., 1962. L'activation de la germination des graines de pin maritime. Rec. Trav. Lab. Biol. Veg. Fac. Sci. Bordeaux. 3. p. 8.

David, R. and A. Guerindon, 1951. Influence stimulante exercée par le froid sur la germination des graines de pin maritime. Comptes Rendus des Seances de la Soc. de biol. et de ses Filialies. Paris 145 (9/10): 715-717.

Donald, D. G. M., 1963. Results of tetrazolium bromide viability tests on seed of three *Pinus* species grown commercially in South Africa. For. in S. Afr. 2: 99-110.

Guitton, Y., 1965. Some physiological effects of the imbibition of *Pinus pinaster* seed. Trav. Lab. For. Toulouse. 1 (6) art. 24. p. 5.

Harding, J. H., 1952. The effect of mechanical dewinging on *Pinus pinaster* seed. Aust. For. 16: 47-52.

Hopkins, E. R., 1960. Germination stimulation in *Pinus pinaster* Ait. Bull. For. Dep. W. Aust. No. 66. p. 10.

Hopkins, E. R., 1966. Influence of storage and fumigation treatments on the mortality of scions of *Pinus pinaster*. Aust. For. 30: 115-124.

Koller, D., A. M. Mayer, A. Poljakoff-Mayler and S. Klein, 1962. Seed germination. Ann. Rev. Plant Physiol. 13: 437-464.

Nyman, B., 1963. Studies on the germination in seeds of Scots pine (*Pinus sylvestris* L.) with special reference to the light factor. Studia Forestalia Sciecica No. 2. p. 164.

Perry, D. H. and E. R. Hopkins, 1967. Importation of breeding material of *Pinus pinaster* Ait. from Portugal. Bull. For. Dep. W. Aust. No. 75. p. 66.

Rowe, J. S., 1964. Environmental preconditioning, with special reference to forestry. Ecology 45: 399-403.

Scott, C., 1962. A summary of information on *Pinus pinaster*. For. Abstr. 23 (1) i-viii and 23 (2) ix-xviii.

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