GERMINATION STIMULATION IN *PINUS PINASTER* AIT

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SUMMARY

THE germinative ability of *Pinus pinaster* Ait. seed imported into Australia from Portugal may be significantly improved by using pre-sowing treatments incorporating both cold soaking and stratification techniques.

In trials carried out in Western Australia in recent years both the rate of germination and the total germination have been significantly increased. Seed treated in 1958 gave 80 per cent. of the total germination in 28 days as compared with a 38 per cent. germination for untreated seed under the same conditions. Comparable values for 1959 trials over a 23 day period were 86 per cent. and 55 per cent. respectively.

The final results of treatments tested were markedly influenced by both the length of the soaking and the length of the stratification technique employed. Most favourable responses have been obtained by stratifying the seed for seven to nine weeks at 36°F. after soaking for eight days in water at room temperature. Drying the seed before sowing was not detrimental to the treatment effect.

Treatment is practical for large quantities of seed with either slight or pronounced dormancy tendencies.

INTRODUCTION

SEED of *Pinus pinaster* Ait. imported from Portugal for plantation establishment in Western Australia, has consistently given poor germination results under local nursery conditions.

The seed has both a slow rate of seedling emergence and a low total germination percentage. Generally, within three weeks of sowing only occasional seedlings can be expected. From three to eight weeks emergence proceeds at a steady low rate to provide a total germination averaging between 50 and 60 per cent. at eight weeks. Under certain circumstances seedling emergence may continue up to 16 weeks in the nursery beds and total germination may range between 40 and 80 per cent.

This behaviour is not restricted entirely to seed received from Portugal. In Western Australia it has been observed with seed batches obtained from French and Corsican sources and with seed of the Portuguese race collected from local stands. David and Guerindon (1952a) indicate that poor germination is also a characteristic of the French races in their native habitats.

In the nursery, the disadvantages associated with this tardy germination are mainly connected with the use of mineral oil weedicides and the incidence of deaths through soil pathogens and drought.

Local nursery practice employs mineral oil weedicides as both pre- and post- emergence sprays (Harding 1952c). The weedicide, to be fully effective, must be applied while the weed crop is in the tender juvenile stage, but at such a time not to burn the emergent pine crop. The time for post emergent spraying is, therefore, determined as that minimum interval following sowing which will allow the pine crop to emerge and harden sufficiently to resist damage from the weedicide.

The eight-week germination period normal to *Pinus pinaster* delays post emergent spray application permitting the weed crop to develop a degree of resistance to the weedicide. Often hand weeding and further spray treatments are required under these conditions.

Nursery deaths ascribed to damping-off and unknown root-rotting pathogens have not been a general problem in local nurseries. In certain years, however, losses due to these causes can be considerable. Gibson (1956) has pointed out the possible increased damping-off risks involved when seed of poor viability is closely sown to obtain reasonably dense seedling populations in the nursery rows. The risk of damage by most soil pathogens and susceptibility to drought must also be increased the longer the seed remains in the soil and the weaker the germinative capacity. Both factors operate against the use of seed with pronounced dormancy tendencies.

During the period 1940-1952 work was initiated by the Western Australian Forests Department to investigate the possibilities of stimulating germination in maritime pine seed to overcome these nursery problems. Numerous pre-sowing trials including hot and cold soaking; mechanical abrasion; chemical treatment with thiourea and potassium nitrate; and both dry and moist cold storage were carried out. Results were generally negative and often confusing. Hot soaking proved lethal in all trials, while cold soaking was either slightly advantageous or had no detrimental effects. Chemical stimulants and mechanical abrasion were ineffective.

Harding (1952b) was able to show that stratification, when not masked by winnowing effects, could be an effective measure against dormancy. David and Guerindon (1952a) have since shown that the germination performance of maritime pine in France can be effectively improved by utilizing a combined pre-soaking and stratification treatment.

In 1958, in the face of recent poor nursery returns, the Western Australian Forests Department decided to follow up the earlier stratification work with the object of developing a standard pre-sowing treatment applicable to the State's annual maritime pine seed requirements. Methods of handling large quantities of seed were adapted from procedure outlined by Wakely (1954) for southern pine seed. Treatment requirements have followed those outlined by David and Guerindon (1952a and b).

Preliminary trials were carried out in 1958 to investigate techniques involved in handling large quantities of seed. Follow up trials in 1959 were designed, on the basis of the 1958 results, to determine the optimum treatment times involved.

MATERIALS AND METHODS

IN 1958, 140 lb. of seed of batch No. 2110 and 10 lb. of batch No. 2109 were used for germination stimulation investigations.

Six treatments of the following soaking and storage combinations were compared:—

(1) Soaking for eight days plus seven weeks' moist cold storage. Seed sown moist.

(2) Soaking for one day plus seven weeks' moist cold storage. Seed sown moist.

(3) Soaking for three hours prior to sowing.

(4) As for treatment (1) with seed dried prior to sowing.

(5) As for treatment (2) with seed dried prior to sowing.

(6) Control. Untreated seed sown dry.

Before soaking, the seed was weighed into 5 lb. lots and stapled into 15 in. x 12 in. calico bags to form a dry pack approximately 12 in. x 12 in. x 1½ in. Each bag was labelled with an aluminium perm-o-tag to indicate the serial number and treatment data.

Soaking was carried out in a large enamel bath, ensuring that the water volume was at least four times the seed volume. One hundred and ten pound of seed was soaked for eight days and 40 lb. was soaked for one day.

Galvanised iron bins of internal diameter 16 in. and depth 2 ft., fitted with a loose lid, were used to hold the seed during storage. A slatted wooden false bottom 3 in. deep was placed in each bin to facilitate bottom drainage and aeration throughout the cold storage period. Hessian covers over these slatted bottoms prevented the packing medium from draining away with excess water.

Clean pine sawdust moistened by 24 hours' soaking and two hours' draining was used as the stratification medium.

Bins were packed to separate the seed bags with at least 2 in. of sawdust enabling 25 lb. of seed to be stored in each bin.

The six bins were placed in cold storage for seven weeks under a temperature range of 35°F. to 38°F. Two temperature tests taken during storage gave readings inside the bins of 36°F.

Slight drying of the sawdust surface was noticed after three weeks and rectified by spraying with cold water.

On removal from cold storage, hand samples taken from each bag for both the eight day and one day soaking lots were grouped and used to commence germination tests for treatments (1) and (2). The remainder of the seed, after drying for 18 hours in an electrically heated unit employed for seed extraction, was used to test treatments (4) and (5).

Treatment (3) was set out for testing at the same time as treatments (1) and (2). Germination tests for the remaining three treatments were started 24 hours later.

All germination testing was on the basis of six replications and a 100 seed unit carried out in sand flats, under glass, with bottom watering. Nursery inspections and counts were also made for treated seed at several nursery centres.

In 1959, after examining the 1958 results, seven treatments covering the following soaking and stratification combinations were tested:—

(1) Control. Untreated seed.
(2) Soaking for eight days.
(3) Soaking for eight days plus stratification for six weeks.
(4) Soaking for two days plus stratification for nine weeks.
(5) Soaking for eight days plus stratification for nine weeks.
(6) Soaking for two days plus stratification for 15 weeks.
(7) Soaking for eight days plus stratification for 15 weeks.

All treated seed was dried before sowing.

Seed used in the 1959 trial was of the single batch serial No. 2367. The treatment procedure employed was identical to that outlined for the 1958 study with the exception that only 4 lb. seed lots were placed in each bag to give a thinner bundle for packing purposes. A total of 40 lb. of seed was used.

RESULTS

Percentage germination values recorded over a 59 day period, in sand flats, for the six treatments employed in 1958 are set out in Table 1 and expressed graphically to compare progressive results after 21, 37, 46 and 59 day germination periods in Figure 1.

Table I.—Mean Percentage Germination Values for Treatments, Serial 2110, 1958.

No.	Treatment	Germination Time, in Days								
		18	22	28	32	37	42	46	53	59
		Percentage Germination								
1	Soaking 8 days + 7 weeks' stratification	13	39	57	62	65	67	69	70	71
2	Soaking 1 day + 7 weeks' stratification	5	24	57	64	69	72	74	75	76
3	Soaking 3 hours	0	3	26	32	41	46	48	51	54
4	Soaking 8 days + 7 weeks' stratification + drying	10	40	55	60	64	70	71	73	74
5	Soaking 1 day + 7 weeks' stratification + drying	3	15	27	38	51	64	69	72	73
6	Control—Untreated	0	9	28	37	45	51	54	59	63

Table II sets out results of an analysis of variance carried out for the germination intervals of 21, 42 and 59 days.

Table II.—Results of Analyses for 1958 Trial.

Treatment	21 days			42 days			59 days		
	Mean	Diff. for significance	F.	Mean	Diff. for significance	F.	Mean	Diff. for significance	F.
1	32·0			67·2			71·5		
2	15·8	0·01 = 11·6		71·3	0·01 = 14·5		76·7	0·01 = 11·0	
3	1·3			47·0			52·5		
4	32·5	0·05 = 8·6	21·8	69·3	0·05 = 10·7	7·4	74·1	0·05 = 8·0	11·0
5	12·2			63·2			73·5		
6	2·3			51·5			62·3		

F at 0·01 = 3·9. F at 0·05 = 2·6.

All stratification treatments markedly increased the germination rate during the early stages of counting; the eight day soaking-stratification combination proved superior in this respect.

The shorter soaking combinations although slow to commence, levelled with the eight day treatment after five weeks with the exception of Treatment (5). Results for this treatment indicate a depressing effect due to drying which is not apparent with the longer soaking.

After eight weeks, all stratification treatments gave a similar total germination of approximately 72 per cent. which proved significantly superior (0.01 level) to the control value of 61 per cent. Treatment (3), soaking without stratification, was inferior to the control at all stages. This depression was significant (0.05 level) only at the eight week count.

Figure I.—Mean Germination Percentages for 21, 37, 46 and 59 Day Germination Periods for 1958 Treatments.

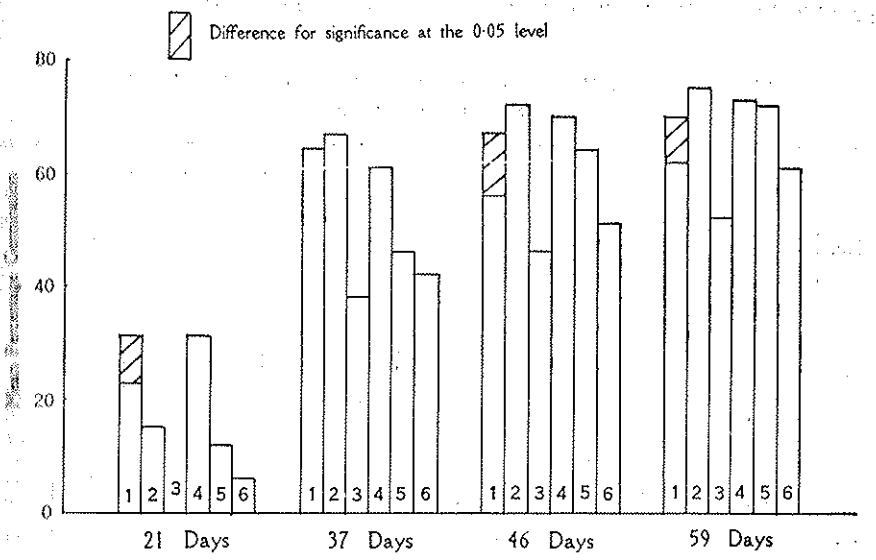
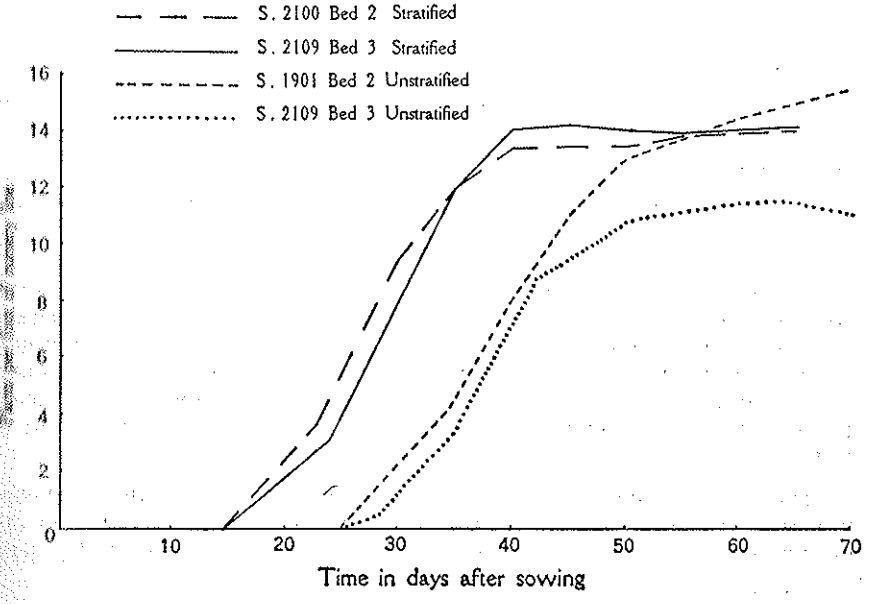


Figure II sets out the progress of counts for treated and untreated seed sown in beds at Gnangara nursery. Results are of a similar trend to those of the controlled germination tests.

Figure II.—Germination of Treated and Untreated Seed in Gnangara Nursery Beds, 1958.



Percentage germination values recorded over a 51 day germination period for the 1959 trial are set out in Table III. These results are expressed graphically for 16, 23, 30 and 51 day intervals in Figure III.

Table III.—Mean Percentage Germination Values for Treatments, Serial 2367, 1959.

No.	Treatment	Germination Time in Days					
		16	23	30	37	44	51
		Percentage Germination					
1	Untreated Seed	16	36	45	49	50	51
2	Soaking 8 days	13	27	36	40	42	43
3	Soaking 8 days + 6 weeks' stratification	30	43	51	55	56	56
4	Soaking 2 days + 9 weeks' stratification	24	36	43	49	50	51
5	Soaking 8 days + 9 weeks' stratification	40	56	62	63	64	65
6	Soaking 2 days + 15 weeks' stratification	16	26	37	43	45	48
7	Soaking 8 days + 15 weeks' stratification	22	32	42	47	49	50

Details of analyses for the 16, 30 and 51 day intervals are contained in Table IV.

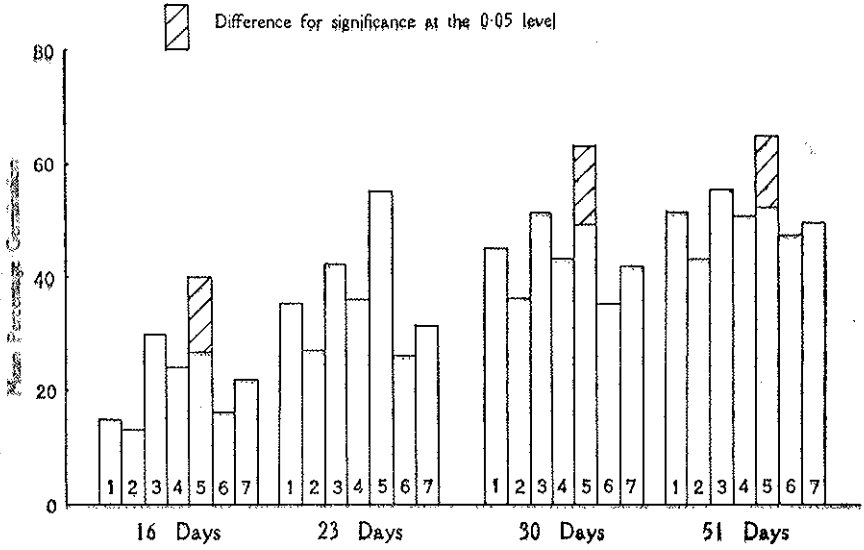
Table IV.—Results of Analyses for 1959 Trial.

Treatment	16 days			30 days			51 days		
	Mean	Diff. for significance	F.	Mean	Diff. for significance	F.	Mean	Diff. for significance	F.
1	15·5			45·3			51·2		
2	12·8	0·01 = 17·5		35·8	0·01 = 18·9		43·0	0·01 = 16·5	
3	30·0			51·2			55·8		
4	24·3		4·6	43·2		3·4	50·5		2·6
5	40·0	0·05 = 13·0		62·0	0·05 = 14·0		64·3	0·05 = 12·2	
6	15·7			36·7			47·5		
7	22·0			42·3			49·5		
		F at 0·01 = 3·5.					F at 0·05 = 2·4.		

Treatment (5) proved the most satisfactory and over the period of testing was responsible for a total germination percentage of 65 per cent. as compared with the control value of 51 per cent. This improvement is significantly superior (at the 0.05 level) to all the others with the exception of Treatment (3). Treatment (3) also increased germination performance over all stages of the testing period but was significantly superior to the control only at the 16 day count.

The longer soaking proved superior to the shorter soaking technique. With the optimum nine week stratification interval (Treatments (4) and (5)) the differences in soaking techniques were significant over the 51 day germination period.

Figure III.—Mean Germination Percentages for 16, 23, 30 and 51 Day Germination Periods for 1959 Treatments.



Soaking alone, without subsequent stratification (Treatment (2)) again depressed the germination capacity as in the 1958 trial. This depression was not significant.

DISCUSSION

In both trials the rate of germination and the total germination was significantly increased as a result of treatment and both the time of soaking and the length of the stratification period employed proved to be important. Most favourable results were obtained with an eight day soak combined with a seven to nine week stratification stage.

Results in the 1958 trial indicated that drying the treated seed prior to sowing had no detrimental effects with an eight day soak, but may have been detrimental with the shorter soaking treatments. This point with respect to a 10 day soaking was established by David and Guerindon (1952b).

In 1959 it was assumed that drying after treatment would have no significant detrimental effects and the treatments for this trial were designed to determine the optimum stratification time while still comparing the short and long term soakings.

David and Guerindon (1952a) found a 10 day soak plus 12 weeks' stratification provided optimum results with French seed and that performance improved with even longer cold storage periods up to 200 days. Figure III shows that under the experimental conditions employed in 1959 the optimum storage period was nine weeks and shorter or longer stratification, with the exception of the six week period, provided significant depressions (at the 0.05 level) from this value. Combining these results with those obtained in 1958, stratification for seven to nine weeks is considered satisfactory until further confirmatory trials can be carried out.

Pre-soaking for eight days proved superior to shorter soaking times in both the 1958 and 1959 trials. In the case of the nine week storage treatments the shorter soaking period significantly depressed (at the 0.05 level) germination over all stages of the 51 days of counting.

The seed of batch No. 2110 used in 1958 showed more pronounced dormancy tendencies than batch No. 2367 employed in 1959. Treatment was, however, effective in both cases although results were not as highly significant with the 1959 seed.

The fact that results have proved favourable with two different seed lots, in two different seasons and that sand flat germination results were duplicated in the nursery beds, indicates that treatment can be adopted as a general prescription. Procedure for treating the seed, as outlined, is satisfactory with the modification of using 4 lb. instead of 5 lb. seed packs to permit improved storage conditions in the bins.

Further trials need to be conducted with different seed batches to determine the most favourable stratification period for all seed which may be received, bearing in mind the difference in behaviour between local and French results.

One deficiency in the trials, which may appear to detract from the work carried out, is the omission of a treatment employing stratification without pre-soaking. This was omitted in 1958 on the basis of Harding's findings in 1950 (unpublished data) that soaking prior to stratification either improved results or had no detrimental effects. The lead offered by David and Guerindon (1952a) also favoured the exclusion of non-soaking treatments. In 1958 the longer soaking period proved superior to shorter periods and the 1959 trial aimed to confirm this result. Direct stratification treatments will, however, be compared in future work.

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