ODC 232.315.3

FORESTRY COMMISSION OF N.S.W.

RESEARCH NOTE No. 14

PUBLISHED February, 1964

THE EFFECT OF PRETREATMENT ON GERMINATION OF SLASH PINE SEED

AUTHOR

W. G. FORREST, B.Sc. For., Dip. For.

(Research Forester, North Coast Research Centre, Coffs Harbour Jetty)

G 46793

ODC 232.315.3

FORESTRY COMMISSION OF N.S.W

RESEARCH NOTE No. 14

PUBLISHED February, 1964

THE EFFECT OF PRETREATMENT ON GERMINATION OF SLASH PINE SEED

AUTHOR

W. G. FORREST, B.Sc. For., Dip. For.

(Research Forester, North Coast Research Centre, Coffs Harbour Jetty)

G 46793—1

PAPERS FOR BRITISH COMMONWEALTH FORESTRY CONFERENCES—

7TH CONFERENCE (Australia and New Zealand)—

Forest Utilisation—Less Valuable Species.
L. S. HUDSON AND P. T. COOKE, 1957

Timber Sales Policy and Procedure .. .. R. J. RITCHIE, 1957

Defoliation of Alpine Ash (*Eucalyptus delegatensis*) by Phasmids.
K. R. SHEPHERD, 1957

Principal Exotic Forest Trees in N.S.W. .. ————, 1957

8TH CONFERENCE (East Africa)—

The Application of Basal Area Control to thinning of *Pinus radiata* Plantations in New South Wales.
S. W. GENTLE, J. L. HENRY AND K. R. SHEPHERD, 1962

TECHNICAL PAPERS—

(This series contains limited numbers of papers, primarily intended for distribution within the Forestry Commission).

*No. 1. Seminar on Management of Low Site Quality Plantations. Moss Vale .. .. ————, 1963

No. 2. Observations on the Growth of Coachwood (*Ceratopetalum apetalum*) in a Selection Forest .. G. N. BAUR, 1963

*No. 3. Seminar on Economics of Forestry, conducted by A. J. Leslie. Bathurst .. .. ————, 1963

No. 4. Pre-emergent Weedicides in Forest Nurseries, Green Hills State Forest .. .. P. A. CASIMIR, 1963

OTHER PUBLICATIONS—

Climatological Basis of Forestry W. A. W. DE BEAUZEVILLE, 1943

Forest Resources, Regions and Trees of N.S.W. ————, 1957

Watershed Management in the United States of America.
K. M. GRAY, 1958

A contribution towards a Watershed Management Research Programme for the Hunter Valley, N.S.W. .. .. K. M. GRAY, 1959

Log Extraction by Crawler Tractor. A report of Logging Operations in Eastern New South Wales .. C. H. GRUGEON, 1961

(Also various publications on forest products issued by the Division of Wood Technology, Forestry Commision of N.S.W.)

*Papers out of print.

THE EFFECTS OF PRETREATMENT ON GERMINATION OF SLASH PINE SEED

W. G. FORREST

INTRODUCTION

The time between sowing of seed and the completion of effective germination is critical for the success of many silvicultural operations. Rapid and even seed germination is important to the nurseryman who aims to produce a uniform crop since delayed germination may upset weeding, tending and tubing programs. When seed is sown direct onto the forest floor it is particularly prone to fungal and insect damage, or to damage and removal by birds and animals during the period before it germinates. Early germination ensures maximum seedling development before the occurrence of unfavourable meteorological conditions.

The rate of pine seed germination can be very greatly influenced by pretreatment, enabling the period for germination to be reduced from several months to only a few weeks. Cold stratification as described by Wakeley (1954) has been used extensively to break seed dormancy and increase the germinative energy of many pine species. For Slash Pine (*Pinus elliottii* Engelmann) seed Wakeley prescribes moist storage at 38° to 41° F. for approximately 30 days.

A number of reports have indicated that a wide variety of alternative methods of pretreatment may increase pine seed germination, and many of these methods appear to be more convenient, more economic, or more effective for use in routine nursery or direct sowing practice than stratification.

Direct sowing operations are controlled closely by changing weather conditions and a pretreatment method that requires only a short duration before sowing would be of considerable advantage. This study was carried out to determine the cheapest means of achieving maximum germination of Slash Pine seed in the shortest treatment time. It involves a laboratory study of several techniques and a field nursery trial of those methods appearing most promising.

LITERATURE

Ching and Parker (1958) found that hydrogen peroxyde (H_2O_2) stimulated the seed germination of *Pseudotsuga, Abies* spp., and *Pinus* spp. when the radicle end of the seed was removed to allow free entry of the solution. Carter and Jones (1962) observed that H_2O_2 penetrated the seed coat of southern pines and that relatively short periods of immersion stimulated germination.

Baur (1961) has reported the American trend towards the "Canadian" method of pretreatment where seed is soaked in water for several hours and then placed in sealed polythene bags for a cold storage period; his report mentioned that the speed of germination was slightly reduced but total germination was comparable to stratification. Baur also referred to a verbal discussion with Mr. Wakeley who considered that this method of pretreatment, althoug being increasingly used at southern pine nurseries, required care as seed can easily lose germinative capacity by prolonged storage at high moisture content.

Gibberellic acid has been shown to stimulate meristematic growth rates on a number of occasions but its effect on seed germination has been varied and inconsistent. Richardson (1959) found germination of Douglas Fir seed to be accelerated by gibberellic acid but total germination was not affected. Grover (1962) showed enhanced germination capacity in Colorado Spruce and Scotch Pine seed following gibberellic acid treatments, but White Spruce seed showed no difference in its germination capacity. Kingard (1961) tested Slash Pine seed germination after soaking in gibberellic acid in concentration from 0 to 800 p.p.m. and showed a final decrease in germinative capacity with increasing concentration of gibberellic acid, the highest germinative capacity being shown by the controls.

Tests conducted by the Eastern Tree Seed Laboratory, Georgia, (U.S. Dept. of Agriculture, 1963) have demonstrated a response in germination of a number of forest tree seeds to treatment with various organic compounds including citric and tartaric acids, and it was suggested that citric acid, as a possible extractor of growth inhibitors, deserved special interest.

It is unlikely that the most economic method of pretreating large batches of Slash Pine Seed will be one involving the use of expensive chemicals. After a review of the literature, the following treatment methods were thought to hold promise for routine use:—

(*a*) Soaking in water.
(*b*) Soaking in hydrogen peroxide.
(*c*) Soaking in water followed by sealed cold storage.
(*d*) Soaking in hydrogen peroxide followed by sealed cold storage.

A laboratory experiment was designed to test these treatment methods each at several levels, and to compare these with cold stratification and untreated control seed.

Laboratory Study

Sufficient Slash Pine seed for the 17 pregermination treatments was counted from a thoroughly mixed lot of clean seed on 23rd March, 1963. Pregermination treatments were commenced and as each treatment concluded a germination test comprising 5 replications each of 10 seeds was laid down. The germination tests were carried out at 80° F., and germinates were counted and removed daily. The test for each treatment was continued for 28 days, after which the remaining whole seeds were counted by cutting and the progressive daily germination as a percentage of the total whole seed was calculated. This last calculation eliminated the variation in total numbers of whole seed between treatments.

Treatments

Soak in H_2O_2—
(*a*) Wash in H_2O_2 30 secs.
(*b*) Soak in H_2O_2 24 hours.
(*c*) Soak in H_2O_2 30 hours.
(*d*) Soak in H_2O_2 36 hours.

Soak in water—
(*e*) Soak in water 24 hours.
(*f*) Soak in water 36 hours.
(*g*) Soak in water 60 hours.

G 46793—2

Soak in H_2O_2 prior to cold store—

(*h*) Soak in H_2O_2 2 hours, cold sealed store 5 days.
(*j*) Soak in H_2O_2 4 hours, cold sealed store 5 days.
(*k*) Soak in H_2O_2 2 hours, cold sealed store 15 days.
(*l*) Soak in H_2O_2 4 hours, cold sealed store 15 days.

Soak in water prior to cold store—

(*m*) Soak in water 8 hours, cold sealed store 5 days.
(*n*) Soak in water 16 hours, cold sealed store 5 days.
(*o*) Soak in water 8 hours, cold sealed store 15 days.
(*p*) Soak in water 16 hours, cold sealed store 15 days.

Controls—

(*q*) Routine cold stratification (moist store 30 days at 30° F.).
(*r*) Untreated control.

Where treatments involved soaking, 50 randomly selected, apparently whole seeds were immersed in 50 mls. of water or of 1 per cent. H_2O_2 as required. For treatments (*h*) to (*p*) the seed was removed from the soak solution after the required period and then placed moist in a prepared sheet plastic bag which was sealed and held at between 38-42° F. for the second period. Seed of treatment (*q*) was held between moist cotton-wool for 30 days at 38-42° F.

Results

The progressive percentage germination of total whole seeds for each treatment has been graphed against time. A separate series of graphs for each of the four pregermination treatment methods is given (Graphs 1-4) and then the duration of treatment which has produced the highest rate of germination within each method is compared with the untreated control and the routine cold stratification (Graph 5).

Soaking in H_2O_2 has increased the total germination over the untreated control in each case and the increase is directly related to the increase in time of immersion. Treatment (*c*) (soaking for 30 hours) has a total germination less than that of treatment (*b*) (soaking for 24 hours) after the 16th day but in fact exceeded that for treatment (*b*) again on the 29th day (not shown on graph).

Soaking Slash Pine seed in water for up to 36 hours has not increased the initial germination rate over the control, but soaking for 60 hours (treatment (*g*)) has doubled the germination percent after 8 days. Again, increasing duration of immersion has increased the total germination, although only the longest soaking of 60 hours has given a final germinative capacity comparable to moist cold stratification.

Both of the "Canadian" methods of treatment, using an initial soaking in H_2O_2 in treatments (*h*) -(*l*) and in water in treatments (*m*)-(*p*), have increased the initial germination rate at all levels of soaking and storing tested. Generally the most prolonged treatments in each case have resulted in the highest germination. With preliminary soaking in either water of H_2O_2, storage for 15 days is necessary to give a final germination equal to that of cold stratification, but there is little advantage in doubling the initial soaking duration of 2 hours for H_2O_2 or 8 hours for water. Treatment (*l*) (soaked in H_2O_2 for 4 hours and then cold stored for 15 days) was the only treatment to result in 100 per cent. germination of the whole seed.

Summary

Slash Pine seed has been subject to pregermination treatments involving soaking in water or hydrogen peroxide, or a short soaking followed by cold storing in sealed plastic bags. The subsequent germination of seed treated in this way has been assessed and compared with the germination of untreated seed and seed which had been cold moist stratified. The germination rates were compared by calculating for each treatment the percentage of total whole seed germinated daily for 28 days.

Cold moist stratification for 30 days has not markedly influenced the initial germination rate; but after 8 days germination of the untreated seed tapered off and reached a maximum of 56 per cent. after 28 days, whereas the stratified seed continued to germinate at an even rate to 97 per cent. in the same time. Germination of the untreated seed would have continued over an extended period.

Total germination comparable to that achieved by cold stratification has also been obtained by soaking seed in water for 60 hours or in 1% hydrogen peroxide for 36 hours, and also by an initial soaking in water for 8-16 hours or peroxide for 2-4 hours followed by cold storage in a sealed plastic packet for 15 days at 40° F. All of these treatments have increased the initial germination rate over that obtained from cold stratification.

The treatments with hydrogen peroxide have given very similar results to those where water was used for a longer period. Carter and Jones (1962) consider that 4-5 gallons of peroxide solutions would be required per pound of seed: this would add about 7s. per pound of seed treated to the cost of treatment.

Maximum germination rates and maximum total germination at minimum costs have been achieved by either soaking Slash Pine seed in water for 60 hours or by soaking in water for 8 hours followed by cold (40° F.) storage in a sealed plastic packet for 15 days.

Field Trial

The laboratory study has indicated that any of the tested pregermination methods could, at the optimum duration of treatment, give germination results comparable with cold stratification. The use of hydrogen peroxide has little advantage over water except that the soaking time can be reduced by a few hours. The cost of treatment weighs against H_2O_2 and the field trial has tested only water treatments.

Routine stratification of seed for sowing at Woolgoolga nursery was commenced on 26th July, 1963. Approximately 50 lb. of seed were placed in four hessian bags and these were placed moist between layers of peat moss and held for 38 days at 40° F. At the time stratification was commenced 15 lb. of seed was taken from the same batch and divided into three lots of 5 lb. for subsequent treatment as follows:—

A. 5 lb. of seed soaked for 60 hours in 5 gallons of water, to finish 8 a.m. on 2nd September, 1963.

B. 5 lb. of seed soaked for 16 hours in 5 gallons of water and then stored for 15 days at 40° F. in a sealed plastic bag, to finish 8 a.m. on 2nd September, 1963.

C. 5 lb. of seed held dry at 40° F. as control.

D. Routine stratification (as described above). Sample removed from bulk lot on 2nd September, 1963.

All treatments were completed by 8 a.m. on 2nd September and each of the batches of seed was dried in the sun before sowing into the prepared nursery beds at Woolgoolga nursery. The layout of the field sowing is shown in figure 1. Four central lines in each of the five consecutive nursery beds were pegged and the four treatments allocated at random within each bed. The first 20 ft. of each line was then sown with the respective treatment using the mechanical seed sower.

Sowing of each bed was completed in a routine manner using stratified seed, and then all beds were subject to the usual watering and weeding treatments.

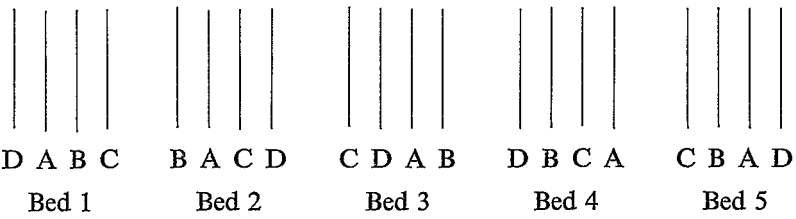


Fig. 1—Layout of Nursery Field Trial.

ASSESSMENT

At a distance 5 feet and 15 feet from the end of each bed at six inch length of each line was pegged. Thus for each treatment a 1-ft. length was marked for assessment along each of the five sown lines.

Beds were inspected daily after sowing and as seedlings germinated within each pegged assessment length they were removed and tallied. Seedlings were classed as germinated and removed when the seedling tip or the attached seed coat was at least half way between ground level and ultimate erect position. The weight of seed sown over the 100-ft. length (20 ft. x 5 beds) of each treatment was calculated by weighing the seed in the sower before and after sowing. Some variation is evident, possibly due in part to changes in seed size and weight after pretreatment, but distribution of seedlings along each line was fairly even. The actual weight of seed distributed was:—

Treatment A (soak 60 hours)	6 oz.
Treatment B (soak and store)	9 oz.
Treatment C (control)	8 oz.
Treatment D (stratify)	9 oz.

It is known from unpublished work that the treatments involved cause an increase in seed moisture content from about 12 per cent. to about 40 per cent.

Very roughly then, the ratio of numbers of seed sown is A : B : C : D = 6 : 9 : 10 : 9.

Results

If the previously calculated ratio of seeds sown in correct and can be applied to the assessment counts, then for comparison of the four treatments the numbers of germinating seedlings in Treatment A, as listed in Table 1, should be increased by 30 per cent. With this amendment then the results of the laboratory study have been substantiated, i.e., the several methods of pretreatment have resulted in a similar total germination value, substantially greater than that for untreated seed.

TABLE 1.—*The progressive number of seedlings germinating within* 10 *weeks of sowing, and the progressive percentage of total germination*

Days after Sowing	Germinated Seedlings				Percentage of Total Germinating Seedlings			
	A	B	C	D	A	B	C	D
20	..	7	..	10	..	4	..	5
30	79	145	31	161	60	82	33	82
40	113	171	74	192	85	97	82	98
50	125	173	80	193	98	98	88	99
60	127	174	93	196	96	99	98	100
70	133	176	94	196	100	100	100	100

The rates of germination, expressed as a progressive percentage of the total number of germinates, for each treatment are shown on Graph 6. Treatment B (16 hour soak and 15 day cold store) and treatment D (stratification) have given identical, high rates of germination. Treatment A (60 hour soak) has resulted in a lower germination rate; about 4 days longer were required to reach 50 per cent. and about 10 days longer were required to reach 80 per cent. of total germination when compared with treatments B and D. The untreated seed (treatment C) has shown a yet lower germination rate, in addition to the fact that the ultimate germination over the 10 week period is about 40 per cent. lower than the total germination value for the several batches of treated seed.

Conclusion

The need for some form of pretreatment to increase pine seed germination rate and to ensure maximum germination within a reasonable period is well known; stratification has been recommended and widely

used, and the benefit of this treatment has been again demonstrated. Several other pretreatment methods have been shown to be as effective, and these include:—

- Soaking in hydrogen peroxide for 36 hours.
- Soaking in water for 60 hours.
- Soaking in hydrogen peroxide for 4 hours, followed by sealed cold storage for 15 days.
- Soaking in water for 8 hours, followed by sealed cold storage for 15 days.

Of these other treatments, the last, seed soaked in water for 8 hours and then stored in a sealed plastic bag at 40° F. for 15 days, has shown results comparable to stratification both in the laboratory and under field conditions. Soaking seed for 60 hours in water gave slightly slower germination in the filed than stratification although the total germination was comparable to, and in the laboratory the rate of germination was better than, that of stratified seed.

Application of Results

None of the treatments tested is superior to stratification for nursery use unless time or space is limited. If routine stratification is not commenced on time, or insufficient seed is stratified, then seed may be pretreated quickly by the "Canadian" method of soaking seed in water for 8 hours and then storing in a sealed plastic bag for about 15 days, or simply by soaking in water for 60 hours.

If it is intended to hasten the germination of seed to be sown direct to the forest floor then full advantage can be taken of favourable weather conditions by holding the seed dry until 2-3 days before sowing is proposed and then immersing the seed in water. The rate of subsequent germination will increase with increasing soaking time up to about 60 hours.

References

Baur, G. N. (1961)—Study Tour of South Eastern United States of America. For. Comm. N.S.W. rep.

Carter, M. C. and Jones, L. (1962)—"The Effect of Hydrogen Peroxide on the Germination of Loblolly and Slash Pine Seed". Sth. east. For. Exp. Sta. pap. 141.

Ching, Te May and Parker, M. C. (1958)—"Hydrogen Peroxide for Rapid Viability Tests on some Coniferous Tree Seeds." For. Sci. 4 (2), 128-134.

Grover, R. (1962)—"Effect of Gibberellic Acid on Seed Germination of Elm, Scotch Pine, Colorado and White Spruce". For. Sci. 8 (2), 187-190.

Kingard, L. R. (1961)—"An Experiment in Treatment of *P. elliottii* Seed with Gibberellic Acid". For. Abstracts 23 (1), item No. 442.

KOZLOWSKI, T. AND GENTLE, A. C. (1959)—"Influence of the Seed Coat on Germination. Water Absorption and Oxygen Uptake of Eastern White Pine Seed". For. Sci. 5 (4), 389-395.

LANQUIST, K. B. (1961)—"Cold Treatment of Seed and Moisture Absorption". For. Abstracts 23 (3), item No. 3489.

RICHARDSON, S. D. (1959)—"Germination of Douglas Fir Seed as Affected by High Temperature and Gibberellic Acid". For. Sci. 5 (2), 174-181.

U.S. Dept. of Agriculture (1963)—"At the Southeastern Forest Experiment Station". Ann. Rep., Stheast. For. Exp. Sta.

WAKELEY, P. C. (1954)—"Planting the Southern Pines". U.S. Dept. of Agriculture Monograph No. 18.

FORESTRY COMMISSION OF N.S.W.
RESEARCH PUBLICATIONS

RESEARCH NOTES—

No. 1. Field Identification of Seedlings of the Major Tree Species in the Blackbutt and Flooded Gum Areas. A. G. FLOYD, 1958

No. 2. Investigations in Sowing of Flooded Gum. A. G. FLOYD, 1960

No. 3. N.S.W. Rainforest Trees. Part 1: Family Lauraceae. A. G. FLOYD AND H. C. HAYES, 1960

No. 4. Raising and Planting of Flooded Gum G. N. BAUR, 1959

No. 5. Nursery Spacing and Grading of Slash Pine Seedlings. G. N. BAUR, 1960

No. 6. The Effects of Low Pruning on Increment in Radiata Pine Plantation .. .. .. K. R. SHEPHERD, 1961

No. 7. N.S.W. Rainforest Trees. Part 2: Families Capparidaceae, Escalloniaceae, Pittosporaceae, Cunoniaceae, Davidsoniaceae .. A. G. FLOYD AND H. C. HAYES, 1961

No. 8. Forest Vegetation in Northeastern New South Wales. G. N. BAUR, 1962

No. 9. Silvicultural Practices in Rainforest of Northern N.S.W. G. N. BAUR, 1962

No. 10. Investigations into the Natural Regeneration of Blackbutt (*Eucalyptus pulularis*) .. .. .. A. G. FLOYD, 1962

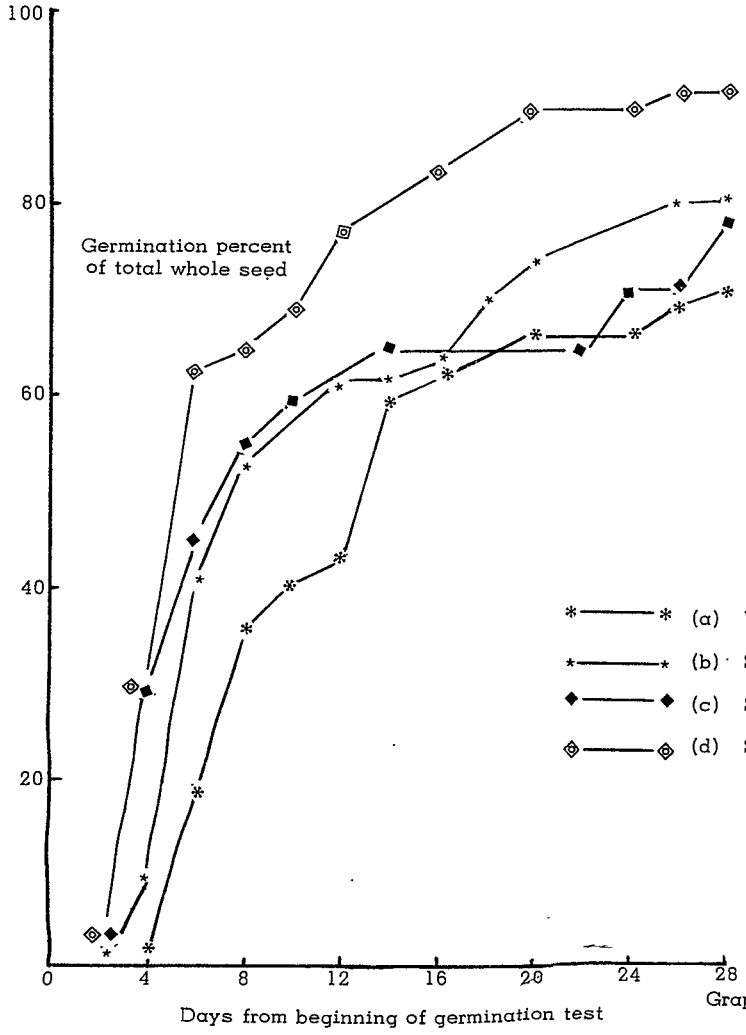
No. 11. Entomoligical Research on the Cause of Mortalities of *Eucalyptus saligna* (Sydney Blue Gum). K. M. MOORE, 1962

No. 12. Insect Attack on *Pinus* spp. .. .. K. M. MOORE, 1962

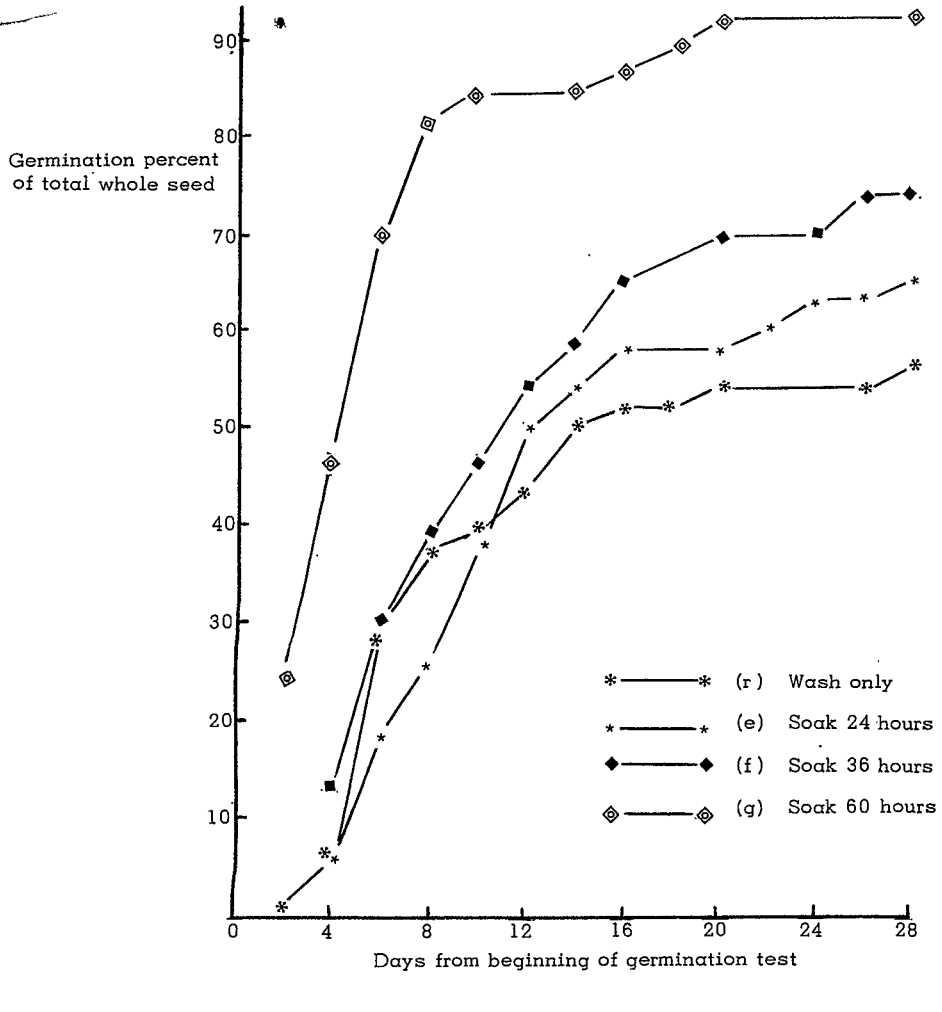
No. 13. The Cypress Pine Sawfly Subspecies: *Zenarge turneri turneri* and *Z. t. rabus* .. K. M. MOORE (in press)

Sydney: V. C. N. Blight, Government Printer—1964

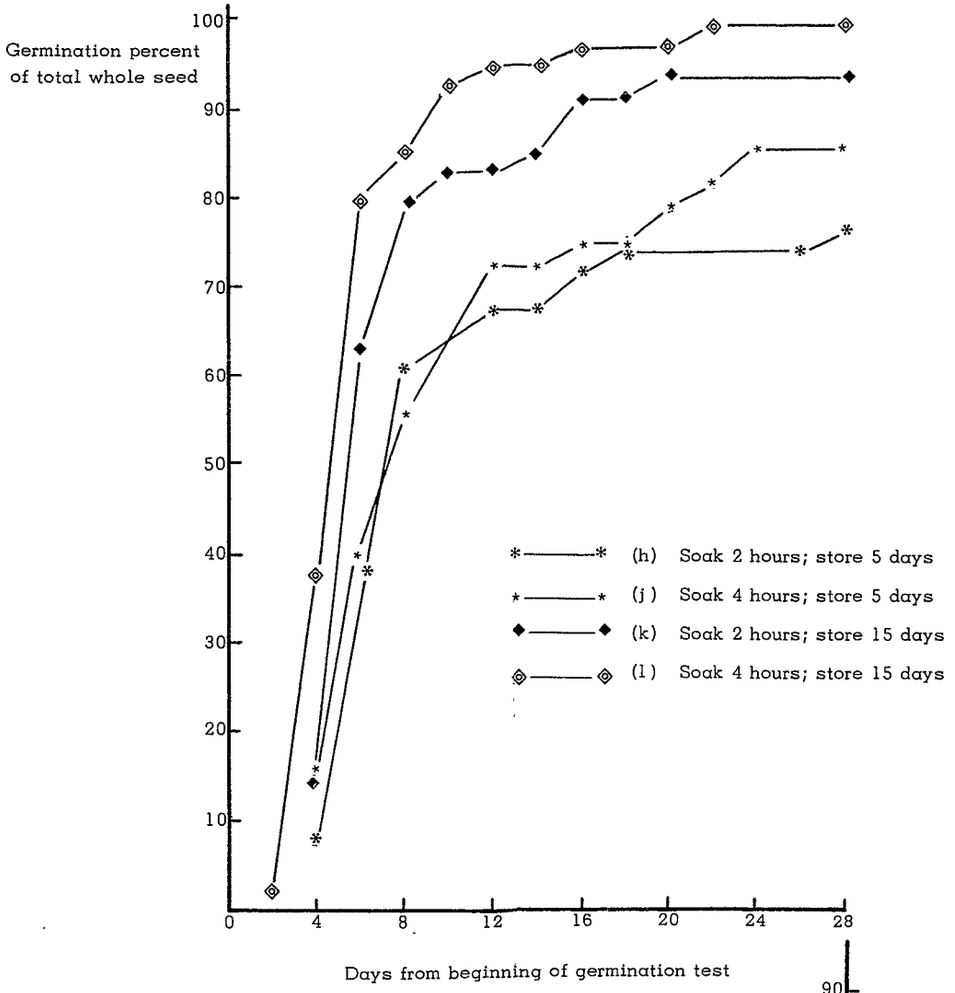
Graph 1 PREGERMINATION TREATMENTS
- SOAK IN HYDROGEN PEROXIDE.



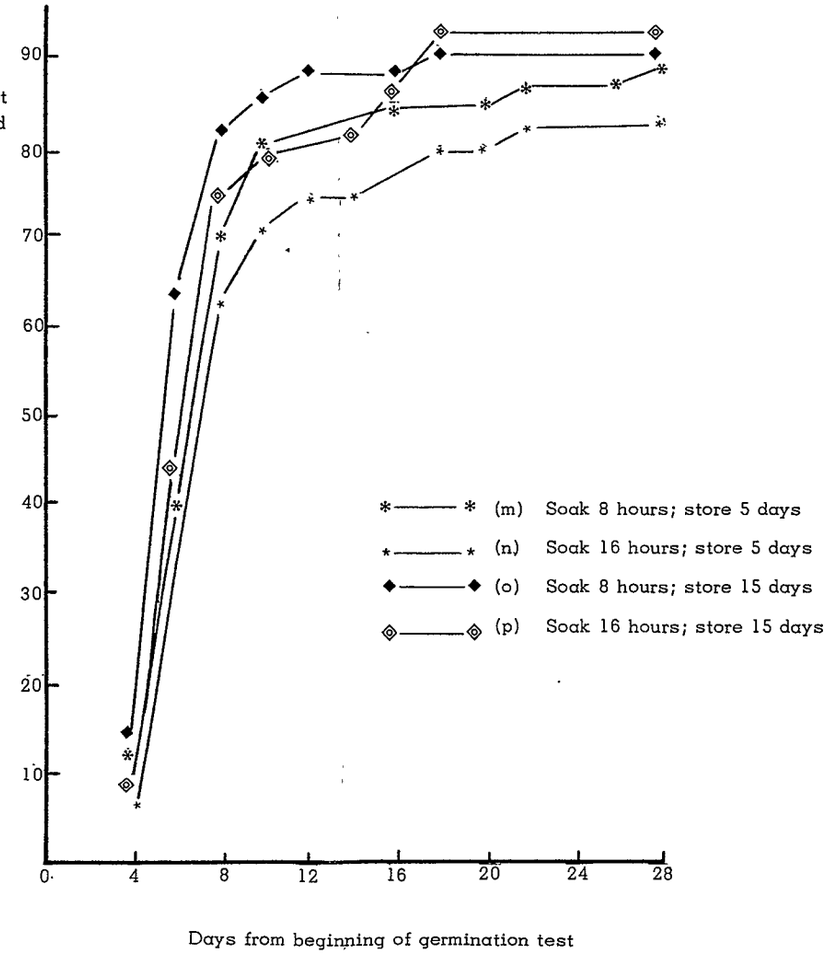
Graph 2 PREGERMINATION TREATMENTS - SOAK IN WATER



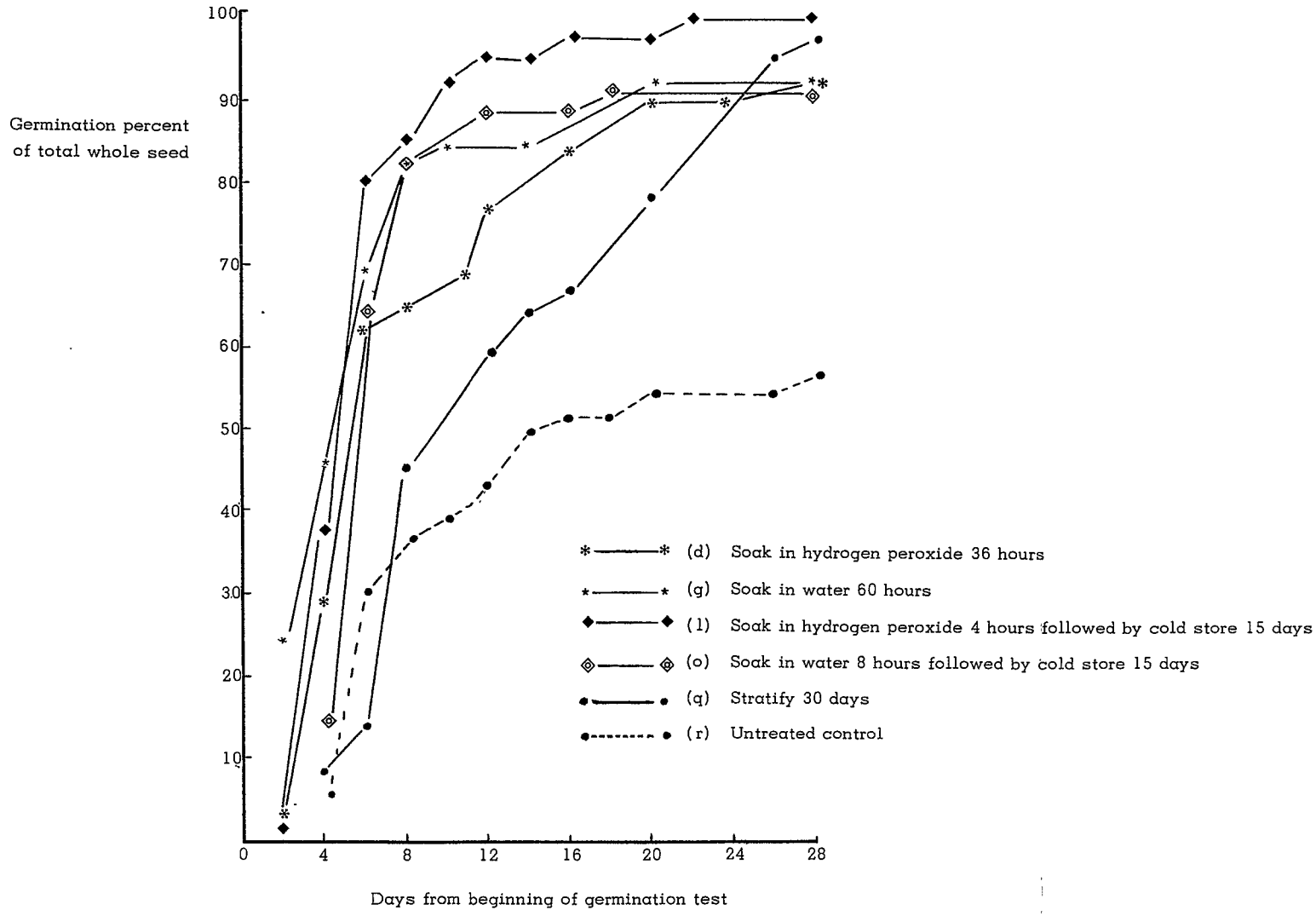
Graph 3 PREGERMINATION TREATMENTS
- SOAK IN HYDROGEN PEROXIDE FOLLOWED BY COLD STORE.



Graph 4 PREGERMINATION TREATMENTS
- SOAK IN WATER FOLLOWED BY COLD STORE.



Graph 5 OPTIMUM PERIOD OF TREATMENT FOR EACH PREGERMINATION METHOD AND COMPARISON WITH STRATIFICATION AND UNTREATED SEED.



Graph 6 RATE OF GERMINATION OF FIELD TRIAL.