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Report Title . Effects of stratification and temperature on the germination of Dalbergia cochinchinensis,
Pinus kesiya and Pinus merkusii

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Coding (see lists overleaf)

Discipline	Region	BioGeo Unit	Species	Report Type
<u>IP</u>	<u>ALL</u>		<u>Pinus</u>	<u>JP</u>

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Coding Structures for use with
Title and Approval Form

DISCIPLINE

GE - Genetics
BR - Tree Breeding
TP - Tree Physiology
BI - Biometrics
EC - Ecology
PE - Pedology and Soils
HY - Hydrology
SI - Silviculture
WI - Wildlife

REPORT TYPE

PA - Problem Analysis
WP - Working Plan
PR - Progress Report
MP - Meeting Proceedings
FP - Final Report
BP - Formal Research Branch Publication
JP - Journal Publication
SR - Special Report - Non E.P. Specific

REGIONS

V - Vancouver
R - Prince Rupert
G - Prince George
K - Kamloops
N - Nelson
C - Cariboo
ALL - More than one region

BIOGEOCLIMATIC UNIT

Use Standard Symbols

From Standard Symbols and Codes, Research Branch Policy, April 1984.

<u>COMMON NAME</u>	<u>SPECIES CODE</u>	<u>BOTANICAL NAME</u>
Douglas-fir	Fd	<u>Pseudotsuga menziesii</u> (Mirbel) Franco
subalpine fir	Bl	<u>Abies lasiocarpa</u> (Hook.) Nutt.
amabilis fir	Ba	<u>Abies amabilis</u> (Dougl.) Forbes
grand fir	Bg	<u>Abies grandis</u> (Dougl.) Lindl.
lodgepole pine	Pl	<u>Pinus contorta</u> Dougl. ex Loud.
western white pine	Pw	<u>Pinus monticola</u> Dougl. ex D. Donn in Lamb.
Ponderosa pine	Py	<u>Pinus ponderosa</u> Dougl. ex Loud. and Laws.
whitebark pine	Pa	<u>Pinus albicaulis</u> Engelm.
Sitka spruce	Ss	<u>Picea sitchensis</u> (Bong.) Carr.
white spruce	Sw	<u>Picea glauca</u> (Moench) Voss
Engelmann spruce	Se	<u>Picea engelmannii</u> Parry ex Engelm.
western hemlock	Hw	<u>Tsuga heterophylla</u> (Raf.) Sarg.
mountain hemlock	Hm	<u>Tsuga mertensiana</u> (Bong.) Carr.
western redcedar	Cwr	<u>Thuja plicata</u> Donn
yellow-cedar	Cy	<u>Chamaecyparis nootkatensis</u> (D. Don) Spach
western larch	Lw	<u>Larix occidentalis</u> Nutt.
alpine larch	La	<u>Larix lyallii</u> Parl.
red alder	Dr	<u>Alnus rubra</u> Bong.

Effects of stratification and temperature on the germination of Dalbergia cochinchinensis, Pinus kesiya, and Pinus merkusii

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ABSTRACT

The effects of stratification and incubation temperature on seed germination were investigated for Dalbergia cochinchinensis, Pinus kesiya, and P. merkusii. Seeds were either soaked for 24 h, or stratified (soaked for 24 h and stored at 2-5°C) for 2 weeks, then incubated at 30°C/20°C, 30°C, or 20°C for 21 days. Germination test results were also compared to results of two other rapid viability tests, X-ray and tetrazolium chloride (TZ).

Total germination of Dalbergia cochinchinensis was greater when seeds received no stratification treatment. Of the three incubation temperatures, germination was most rapid when seeds were incubated at 30°C, but highest when seeds were incubated at 30°C/20°C. Germination test results agreed well with results of X-ray tests, but poorly with TZ tests.

Stratification enhanced germination rates of Pinus kesiya and P. merkusii under all incubation temperatures. Germination was most rapid when the seeds were incubated at 30°C, and slowest when incubated at 20°C. Results of X-ray and TZ tests corresponded well with results of germination tests of P. kesiya, but correlated poorly with germination tests of P. merkusii.

Germination of P. merkusii was low under all treatments, and longer stratification is suggested. Further research is needed to improve germination performance in this species.

INTRODUCTION

Seeds are considered dormant when they are placed under conditions favourable for growth, yet fail to germinate. Dormancy, found in many tree seeds, is an important mechanism to enhance survival by delaying germination until conditions in the external environment are conducive to active growth (Osborne 1981). The expression of dormancy is under genetic control (Naylor 1983), but it is also strongly influenced by environmental factors (Steinhoff *et al.* 1983; Rehfeldt 1983, 1985).

Many important tropical forest trees are legumes, of which Dalbergia cochinchinensis is representative. For most leguminous seeds, the inability to germinate is due to the impermeability of the seed coat (Barton 1947, Brant *et al.* 1971, Ballard 1973, Royston 1978, Werker 1980). Although hand or mechanical scarification, acid treatments, and water treatments are commonly used to break dormancy of hard-coated leguminous seeds, (Willan 1985) scarification was not deemed necessary for D. cochinchinensis because its seeds absorb water readily (Kobmoo *et al.* 1990a). Seeds of conifers, however, often require stratification, i.e., chilling of moist seeds at 2-5°C for several weeks to several months, to break dormancy and ensure complete germination. Since stratification increases germination rates as well as total germination, seedling emergence is completed within a shorter period (Allen 1960, 1962; Edwards 1973). Another advantage of stratification is that it enhances the ability of seeds to germinate under a wide range of temperatures (Leadem 1989). Nursery managers find stratification especially attractive because it enhances their ability to manage for the variety of different environmental conditions which may exist in seedling nurseries (Leadem 1989).

Tree seed quality is usually assessed by means of a standard germination test requiring a minimum of three weeks; with stratification, the test can take six weeks or longer.

Sometimes the standard test cannot be performed because of time constraints or mechanical or physiological dormancy, and in such instances, "quick tests" of seed viability can provide reasonably good estimates of seed quality (Leadem 1984). Quick tests such as the X-ray and tetrazolium chloride (TZ) provide an estimate of viability within several hours. Since quick tests are performed independently of environmental factors, they have an advantage over the usual germination tests because seed quality is assessed irrespective of dormancy status.

The X-ray test is based upon the physical examination of the embryo and endosperm. Although X-radiography can rapidly distinguish good from defective seeds (Kamra 1976), the technique has had relatively limited application in tropical forest seeds, especially in southeast Asia (Kobmoo *et al.* 1990b). X-radiography can be used to detect disease-infested seeds (Kamra 1974), mechanically-damaged seeds (Kamra 1973, Singh and Banerjee 1968), and internal insect infestation (Freeman 1965, Yates 1972, 1974). The X-ray test has been demonstrated to be a reliable indicator of seed viability, showing good correlations with germination test results (Leadem 1981). The use of "soft" (low energy) X-radiation does not affect seed germination or result in any apparent chromosome damage (Kamra and Simak 1965), and is also safer for operators working in seed testing laboratories.

The TZ technique defines live and dead areas of the embryo and endosperm by differential, topographic staining. When seeds are incubated for several hours in tetrazolium chloride solution, dehydrogenase enzymes in actively respiring areas of the seed react with the colourless TZ to form the red-coloured formazan, which precipitates in and stains the

tissues. By evaluating the location and intensity of the formazan stain, one can readily differentiate between healthy and damaged portions of the seed.

The TZ evaluation focuses directly upon the physical and physiological condition of the embryo and endosperm (or, in conifers, the megagametophyte), but relies heavily upon the expertise and intuition of the experienced analyst (Leadem 1984). Since the TZ rating is independent of inhibitions imposed by dormancy, results may not relate well to actual performance. TZ viability ratings are often higher than actual germination in standard incubator tests (Kaul and Zentsch 1969; Simak 1970, Leadem 1984). However, incubator tests are usually more relevant to nurseries because results correlate directly to nursery performance. The utility of the test depends upon whether the analyst is interested in ultimate seed potential, or in assessing seed performance under field conditions.

The purpose of this study was to investigate the effects of different incubation temperature regimes (30°C/20°C, 30°C, and 20°C) on seed germination of Dalbergia cochinchinensis, Pinus kesiya, and P. merkusii, and to determine how stratification affected the germination response. Seed viability predicted by the X-ray and tetrazolium chloride (TZ) quick tests was compared to germination test results to determine their suitability for testing these tropical seeds. It is hoped that the information from this study will facilitate nursery operations in tropical countries where Dalbergia cochinchinensis, Pinus kesiya, and P. merkusii are grown.

MATERIALS AND METHODS

Seed sources

Seeds were obtained from the ASEAN-Canada Forest Tree Seed Centre, Muak Lek, Saraburi province, Thailand, and the Silviculture Division, Royal Forest Department, in Bangkok. Species and seed sources are listed in Table 1.

Treatments and germination tests

Seeds of each species were divided into two equal groups which were either (1) soaked for 24 h, or (2) soaked for 24 h, drained and then stratified for 2 weeks at 2°C-5°C. After soaking or stratification, seeds were incubated for 3 weeks in controlled environment chambers at (1) 30°C for 8 h and 20°C for 16 h, (2) 30°C for 24 h, and (3) 20°C for 24 h. Light was provided for 8 h daily at a light intensity of about 50 microeinsteins m⁻² sec⁻¹. For the alternating temperature regime, light was given during the high temperature period.

For laboratory germination tests, four replications of 50 seeds were used for each treatment. Seeds were placed in covered plastic boxes (12 X 12 X 3 cm) on a substrate of one layer of Kimpak and two layers of Whatman No. 1 filter paper, moistened with 50 mL de-ionized water. Germination counts were made daily and terminated after 3 weeks. Seeds were considered germinated once the radicle and hypocotyl had grown to four times the length of the seed coat.

X-ray tests

Each replicate of 50 seeds used for the germination tests was X-rayed to determine whether seeds were empty or filled. Settings for the Faxitron X-ray machine were 3 mA and 15 kV, and exposure time was 2 minutes. Exposures were processed in a Kodak Industrex Instant Processor using light sensitive paper, 8 X 10 inches (20.3 X 25.4 cm) in size, to make the radiographic images. Seed development and viability were determined by evaluating the density, shape and location of opaque matter (bright areas) seen in the X-rays.

Tetrazolium chloride tests

The TZ solution (pH 6.5-7.0) was prepared from 2,3,5-triphenyl tetrazolium chloride, diluted to 1% (W/V) in distilled water, and stored at 5°C in the dark. To determine the most suitable length of time to stain seeds in the TZ solutions, seeds of Dalbergia cochinchinensis, Pinus kesiya, and P. merkusii were incubated in the dark at 37°C for various periods from 1 to 24 h (Table 3). Because of the limited amount of seed material, only two replications of 5 seeds were used for each treatment in these preliminary trials. When staining was complete, seeds were immediately rinsed two to three times with distilled water. The period required for adequate staining varied with each species, but light pink to red staining of the embryo was favoured because seeds are easier to evaluate when tissues are less heavily stained (Leadem 1984).

To prepare the seeds for TZ tests, Pinus seeds were soaked intact for 24 h in deionized tap water, but for Dalbergia cochinchinensis, it was necessary to cut off 1/6 of the seed at the end opposite the embryo. The seeds were then incubated in 1% TZ solutions at

37°C in the dark. Dalbergia cochinchinensis seeds were incubated for 24 h, and Pinus kesiya and Pinus merkusii seeds were incubated for 4 h, the periods previously found to be most effective (Fig. 1). Four replications of 20 seeds were used for each TZ viability test. Seed viability was evaluated by observing the intensity and location of staining, and categorizing each seed according to the four quality classes defined in Table 4.

Data analysis

Treatments and replications were completely randomized for each species in the germination test. Total germination after day 21, and the viable seeds as evaluated by the X-ray and TZ tests were calculated as percentages based on the number of seeds used. Germination data were analyzed by means of ANOVA and statistical differences were determined at 5% probability level. Means were compared using Duncan's multiple range test (Duncan 1965). The T-test at $p = 0.05$ was performed to test the differences between total germination and the viability of seeds determined by the X-ray and TZ tests.

RESULTS AND DISCUSSION

Dalbergia cochinchinensis

Germination generally started 5 to 6 days after sowing, except for seeds which were incubated at 20°C (Figs. 3,4,5). Unstratified seeds started to germinate on day 15, while seeds stratified for 2 weeks did not germinate at all. The speed of germination also varied,

depending on whether the seeds were stratified, and the temperature at which seeds were incubated (Figs. 3,4). Stratification did not appreciably improve the rate of germination under any of the three temperature regimes. The fastest rates were obtained with 30°C and no stratification, and the slowest rates were achieved with 20°C and 2 weeks' stratification. Under alternating 30°C/20°C, rates of stratified seeds were slightly faster.

The effects of stratification on total germination were not apparent after day 14 for seeds incubated under 30°C/20°C and 30°C (Fig. 5). Under 30°C/20°C, total germination of unstratified seeds was initially slower, but ultimately greater than that of all other treatments. Stratified seeds at 30°C germinated quickly initially, but achieved the lowest total germination. Highest germination was achieved by unstratified seeds incubated under 30°C/20°C and 30°C, the next highest germination was found for stratified seeds incubated under 30°C/20°C and 30°C. Almost no germination occurred for stratified and unstratified seeds incubated under 20°C. Total germination after day 21 ranged from no germination for seeds stratified for 2 weeks at 20°C to 96.5% for unstratified seeds incubated at 30°C/20°C (Table 5). Analysis of variance showed that germination differed significantly ($p = 0.01$) among the various treatments (Table 5).

To ascertain whether quick tests could be used to predict viability of Dalbergia cochinchinensis, a comparison was made between total germination under the best test conditions (no stratification at 30°C/20°C), and the percent of viable seeds determined from X-rays and the TZ test (Fig. 2). Using the T-test, significant differences ($p = 0.01$) were found between germination test and TZ test results, but no significant differences between the germination and X-ray tests. For the rapid determination of seed quality, X-ray test results

(97%) agreed well with actual germination (96.5%) of Dalbergia cochinchinensis. This is reasonable considering that X-ray evaluation works best in species which exhibit no dormancy (Leadem 1984). The TZ test (72.5%) showed poorer correspondence with germination tests. This may be due to impermeability of the seed coat which could impede uptake of the TZ solution.

Pinus kesiya

Germination of stratified seeds started on days 4, 5, and 8 for seeds which were incubated at 30°C, 30°C/20°C, and 20°C, respectively. Unstratified seeds commenced germination on days 5, 6, and 9 when incubated at 30°C, 30°C/20°C, and 20°C (Fig. 6). The 2-week stratification treatment enhanced the germination rate at all three incubation temperatures (Fig. 6). The germination rate was highest on day 6 (42%) under 2-week stratification at 30°C (Figs. 6,7). The slowest germination was observed for unstratified seeds incubated at 20°C.

Germination was almost complete by day 10 for seeds which were incubated at 30°C and 30°C/20°C (Fig. 8). Seeds incubated at 20°C were much slower, however, and continued germination until day 16. By day 21, total germination of all treatments was approximately equal, ranging from 91% to 96% (Table 5). Analysis of variance and Duncan's multiple range test indicated no significant differences between germination of stratified seeds incubated at 30°C/20°C and 30°C and 20°C, and unstratified seeds incubated at 30°C. There was a significant difference in total germination of unstratified seeds incubated at 30°C/20°C and 20°C, but the value was not large enough to be of practical importance.

To evaluate the efficacy of viability tests, a comparison was made of total germination under the best treatment conditions (2-week stratification at 30°C incubation temperatures), and viable seeds evaluated from X-ray and TZ tests. Using the T-test at a $p = 0.05$ level (Fig. 2), no significant differences were found between the three methods.

In summary, stratification enhanced germination rates of Pinus kesiva under all incubation temperatures. Germination was most rapid when the seeds were incubated at 30°C, and slowest when incubated at 20°C. Total germination was the same under all temperature conditions. Results of the X-ray test (93.5%) and the TZ test (90%) corresponded well with the results of germination tests (96.0%).

Pinus merkusii

Germination started on days 7, 8, and 14 for stratified seeds, and on days 8, 9, and 15 for unstratified seeds incubated at 30°C, 30°C/20°C, and 20°C, respectively (Figs. 9,10,11). The germination of stratified and unstratified seeds incubated at 30°C/20°C and 30°C was nearing completion by day 21. However, under 20°C incubation temperatures, germination was still not complete by the end of the test (Fig. 11).

After 21 days, total germination from all treatments ranged from 24.5% to 41% (Table 5). Statistical analysis using ANOVA and Duncan's multiple range test showed significant differences in total germination between the stratified seeds incubated at 30°C and 20°C, but no differences between any of the other treatments.

A comparison was made to determine the correspondence between total germination under the best treatment conditions (2-week stratification at 30°C) and viable seeds evaluated

from the X-ray and TZ tests (Fig. 2). Results of the T-test indicated a significant difference between the germination and TZ tests ($p = 0.05$), and between the germination and X-ray tests ($p = 0.01$). Pinus merkusii appears to be a dormant species, and for dormant seeds, X-rays tend to have poor predictive ability because the visual evaluations of physical characteristics are made irrespective of the dormancy status of the embryo (Leadem 1984).

In general, stratification promoted germination in Pinus merkusii under all temperature regimes. Among the three incubation temperatures, germination was highest at 30°C and lowest at 20°C. From the shape of the germination curves, it seems that the time required for germination testing at 20°C should be longer than 3 weeks. There may even be a case for lengthening the test at 30°C, but lengthening the length of stratification would probably prove to be more beneficial. Further research should be conducted to determine how to improve germination performance in this species.

For rapid determination of seed quality, the X-ray test (94%) indicated higher seed viability than did the results of the germination test (41%) (Fig. 2). The TZ test (26%) underestimated viability, but the results corresponded more closely with the results of the germination test. However, germination was relatively low under the conditions used in this study. Improved methods for treating seeds, such as longer stratification, would undoubtedly alter the correlations between X-ray, TZ, and germination tests.

LITERATURE CITED

- Allen, G.S. 1960. Factors affecting the viability and germination behaviour of coniferous seed. IV. Stratification period and incubation temperature, Pseudotsuga menziesii (Mirb.) Franco. For. Chron. 36: 18-29.
- 1962. Factors affecting the viability and germination behaviour of coniferous seeds. VI. Stratification and subsequent treatment, Pseudotsuga menziesii (Mirb.) Franco. For. Chron. 38: 485-496.
- Ballard, L.A.T. 1973. Physical barriers to germination. Seed Sci. Technol. 1: 285-303.
- Barton, L.V. 1947. Special studies on seed coat impermeability. Contrib. Boyce Thompson Inst. 14: 355-362.
- Brant, R.E., McKee, G.W., and Cleveland, R.W. 1971. Effect of chemical and physical treatment on hard seed of Penngift Crownvetch. Crop Sci. 11: 1-6.
- Duncan, D.B. 1965. A Bayesian approach to multiple comparisons. Technometrics 7: 171-222.
- Edwards, D.G.W. 1973. Effects of stratification on western hemlock germination. Can. J. For. Res. 3(4): 522-527.
- Freeman, C.C. 1965. Criteria for radiographic examination of internal insect infestation. J. Assoc. Official Agric. Anal. Chem. 48: 1183-1185.
- Kamra, S.K. 1973. The use of X-ray radiography for detecting mechanical damage and internal insects in seeds. IN: International Symp. on Biol. of Woody Plants. Slovak Acad. Sci., Bratislava, Yugoslavia, pp. 273-277.
- 1974. X-ray radiography of tropical forest seed. IN: Proc. Seed X-ray Symposium, Macon, GA., USA, pp. 1-20.
- 1976. Use of X-ray radiography for studying seed quality in tropical forestry. Stud. Forestal. Suec. 131, 34p.
- and Simak, M. 1965. Physiological and genetical effects on seed of soft X-rays used for radiography. Botankiska Notiser 118: 254-264.
- Kaul, M.L.H. and Zentsch, W. 1969. Preliminary report about TTC-testing for forest tree seeds in India. Beiträge zur Tropischen und Subtropischen Landwirtschaft und Tropenveterinämedizin, Leipzig (1969) 7(3): 285-289. (For. Abstr. 31, 1970, No. 6268).

Kobmoo, B., Chaichanasuwat, O. and Pukittayacamee, P. 1990a. A preliminary study on pretreatment of seed of leguminous species. *Embryon*, 3(1): 6-10.

----- 1990b. Preliminary studies of x-radiography for seed quality testing of Acacia auriculiformis A. Cunn. ex Beth. seeds. *Embryon*, 3(1): 20-28.

Leadem, C.L. 1981. Quick methods for determining seed quality in tree seeds. IN: Proc. "High-quality collection and production of conifer seed", Nov. 14, 1979, Edmonton, Alberta. Northern For. Res. Centre, Can. For. Serv., Info. Rep. NOR-X-235, pp. 64-72.

----- 1984. Quick tests for tree seed viability. B.C. Min. For. Land Manage. Rep. No. 18. 45 p.

----- 1989. Stratification and quality assessment of Abies lasiocarpa seeds. Can. For. Serv./B.C. Min. For. FRDA Rep. No. 95. 18 p.

Naylor, J.M. 1983. Studies on the genetic control of some physiological processes in seeds. *Can. J. Bot.* 61: 3561-3567.

Osborne, D.J. 1981. Dormancy as a survival stratagem. *Ann. Appl. Biol.* 98: 525-531.

Rehfeldt, G.E. 1983. Adaptation of Pinus contorta populations to heterogeneous environments in northern Idaho. *Can. J. For. Res.* 13: 405-411.

----- 1985. Ecological genetics of Pinus contorta in the Wasatch and Uinta Mountains of Utah. *Can. J. For. Res.* 15: 524-530.

Rolston, M.P. 1978. Water impermeable seed dormancy. *Bot. Rev.* 44: 365-396.

Simak, M. 1970. Germination analyses of Abies alba seed. IN: Proc. Int. Seed Testing Assoc. 35(2): 361-367.

Singh, A., and Banerjee, S.K. 1968. Detection of mechanical damage in soya-bean (Glycine max) seed by radiographic analysis. *Curr. Sci.* 37(11): 322-323.

Steinhoff, R.J., Joyce, D.G., and L. Fins. 1983. Isozyme variation in Pinus monticola. *Can. J. For. Res.* 13: 1122-1132.

Werker, E. 1980. Seed dormancy as explained by the anatomy of embryo envelopes. Impermeability to water, impermeability to oxygen, and mechanical resistance to radicle protrusion. *Israel J. Bot.* 29: 22-44.

Willan, R.L. 1985. A guide to forest seedling handling with special reference to the tropics. FAO Forestry Paper 20/2, 379 p.

Yates, H.O. 1972. Use of radiography in entomology. IN: Proc. Southeastern forest radiography workshop, Athens, GA, USA., pp. 102-130.

----- 1974. Radiography for detection and study of insects in plant seeds. IN: Proc. Seed X-ray Symposium, Macon, GA, USA, pp. 65-78.

Table 1. Species, seed sources, and collection dates

<u>Species</u>	<u>Seed Sources</u>	<u>Collection Dates</u>
<u>Dalbergia cochinchinensis</u>	Kang Koi, Saraburi	Sept. 7, 1990
<u>Pinus kesiya</u>	Samreng, Chiangmai	January 1987
<u>Pinus merkusii</u>	Tung Phaya, Phitsanulok	April 23, 1986

Table 2. Experimental factors used in the germination test.

<u>Factors</u>	<u>Levels</u>
Species	1. <u>Dalbergia cochinchinensis</u> 2. <u>Pinus kesiya</u> 3. <u>Pinus merkusii</u>
Stratification	1. 24 h water soak, no stratification 2. stratification for 2 wk
Temperature	1. incubated at 30°C/20°C for 8 h/16 h 2. incubated at 30°C for 24 h 3. incubated at 20°C for 24 h
Replication	4 replications of 50 seeds per treatment

Table 3. Seed treatments for tetrazolium testing

<u>Species</u>	<u>Soaking¹ period (h)</u>	<u>Seed preparation</u>	<u>Staining² periods (h)</u>
<u>Dalbergia cochinchinensis</u>	24	Cut off 1/6 of seed at end opposite to embryo ³	1,2,4, 6,24
<u>Pinus kesiya</u>	24	Cut longitudinally beside embryo	1,2,4,6
<u>Pinus merkusii</u>	24	Cut longitudinally beside embryo	1,2,4,6

¹ Soak in water at room temperature (20°C-25°C).

² Using a 1% TZ solution (pH 6.5-7.0) at 37°C in the dark.

³ Seeds were cut prior to 24 h water soak; Pinus seeds were cut after 24 h soak.

Table 4. Quality classes for evaluating the tetrazolium test

<u>Class</u>	<u>Description</u>	<u>Viability</u>
I	Embryo completely stained	Viable
II	Very pale staining, or >75% embryo stained	Viable
III	Radicle and/or cotyledon unstained, or >25% of embryo unstained	Non-viable
IV	Embryo unstained	Non-viable

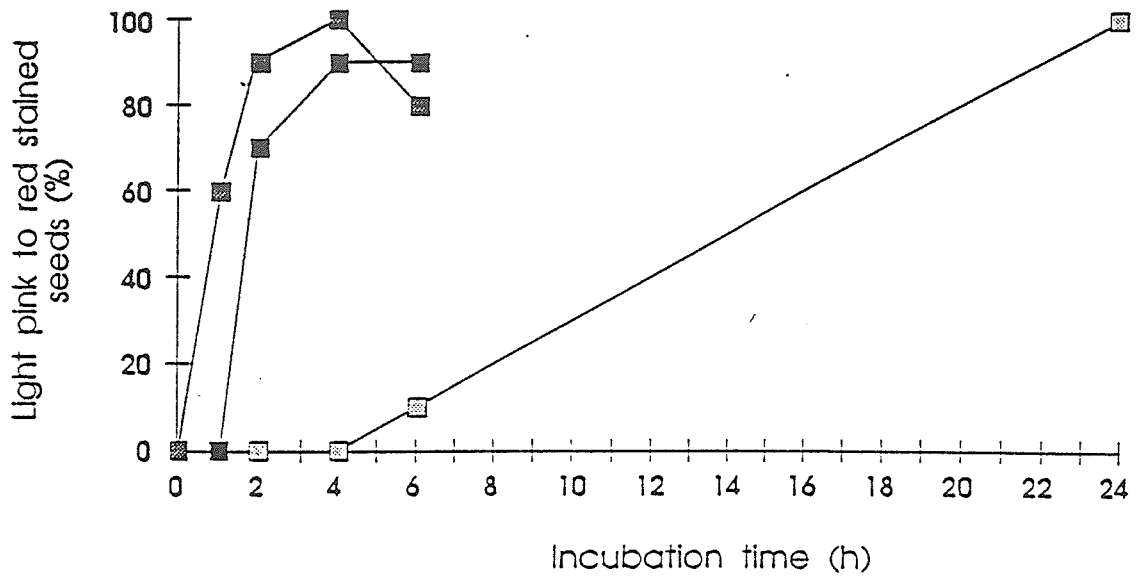
Table 5. Effects of stratification and incubation temperature on germination capacity of Dalbergia cochinchinensis, Pinus kesiya, and Pinus merkusii.

Treatment		Germination capacity (%)		
		<u>Dalbergia</u> <u>cochinchinensis</u>	<u>Pinus kesiya</u>	<u>Pinus merkusii</u>
<u>Strat.</u>	<u>Incubation</u>			
None	30°C/20°C	96.5 a	91.0 b	37.0 ab ²
None	30°C	91.0 a	93.5 ab	37.5 ab
None	20°C	4.5 d	96.0 a	28.0 ab
2 wk	30°C/20°C	88.0 bc	94.0 ab	37.5 ab
2 wk	30°C	83.0 c	92.0 ab	4.1 a
2 wk	20°C	0.0 d	92.0 ab	24.5 b

¹ Significance level determined from F-values of ANOVA, ns = non-significant; ** = significant difference at $p = 0.01$).

² Values followed by the same letter in each column are not significantly different at $p = 0.05$ as determined by Duncan's multiple range test (1965).

Figure 1. Light pink to red-stained seeds of Dalbergia cochinchinensis, Pinus kesiya and Pinus merkusii as a percentage of the total.



—□— *Dalbergia cochinchinensis*

—▨— *Pinus kesiya*

—■— *Pinus merkusii*

Figure 1

Figure 2. Comparison of total germination at the suitable treatment (%) and comparison of viable seeds evaluated from X-ray (%) and the TZ test (%) for each species.

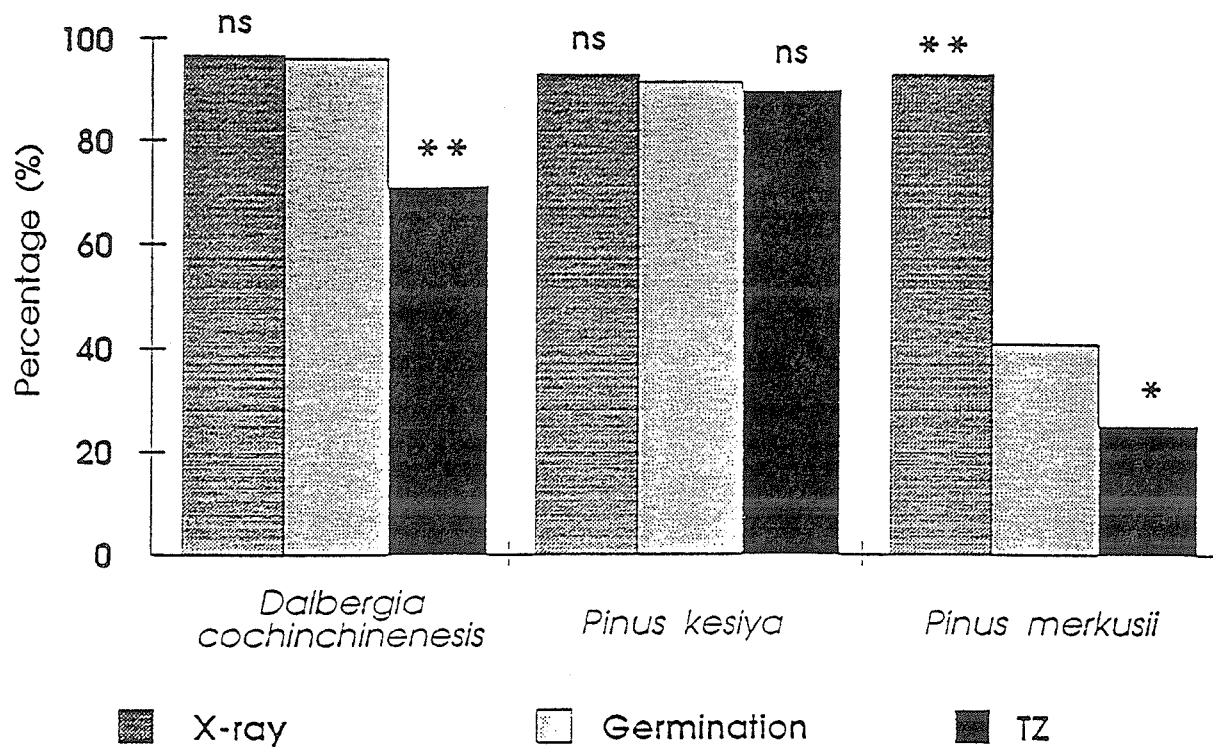


Figure 2

Figure 3. Effects of stratification on the germination rate of Dalbergia cochinchinensis seeds at three incubation temperatures.

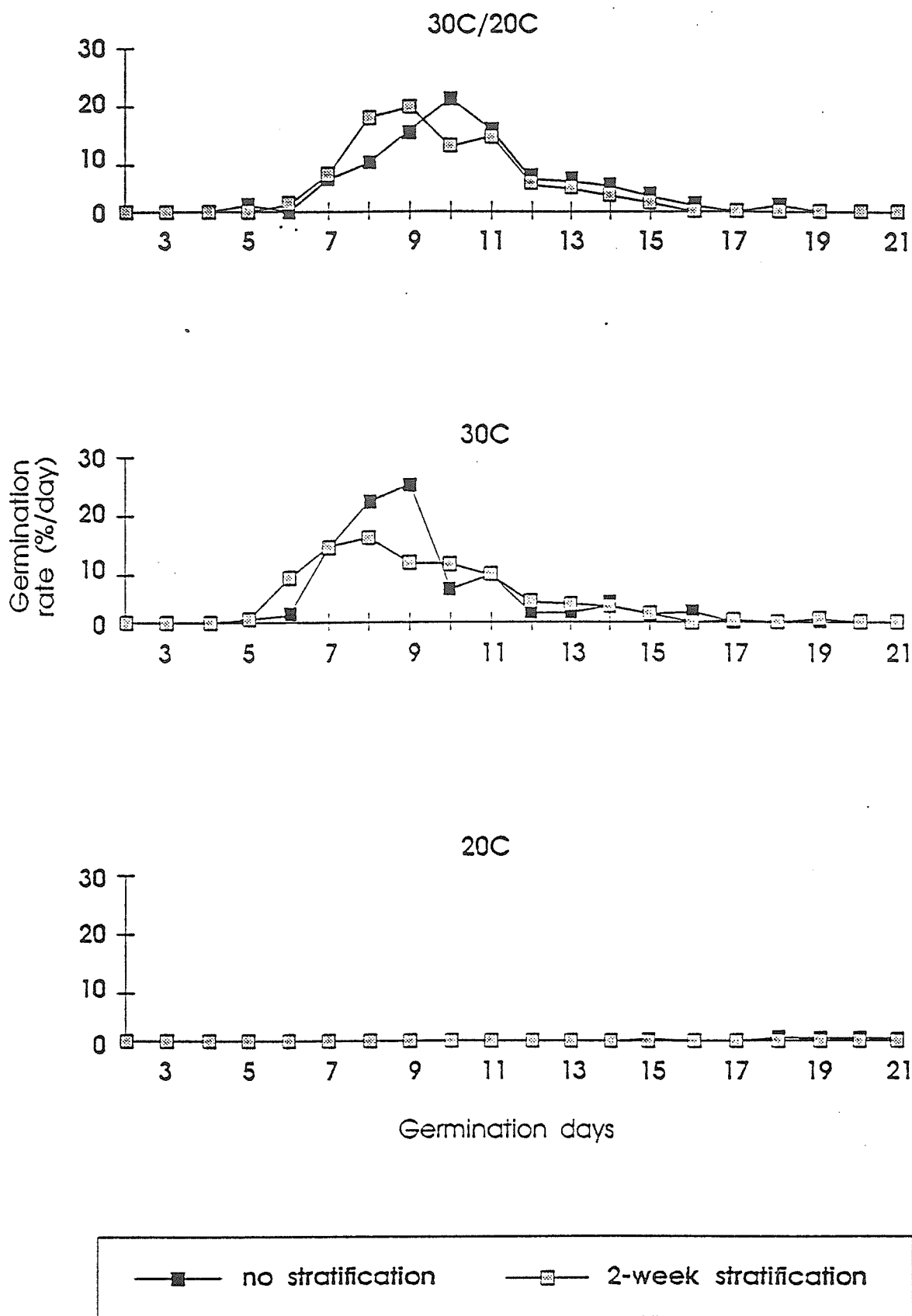


figure 3

Figure 4. Effects of incubation temperature on the germination rate of Dalbergia cochinchinensis seeds at two stratification levels.

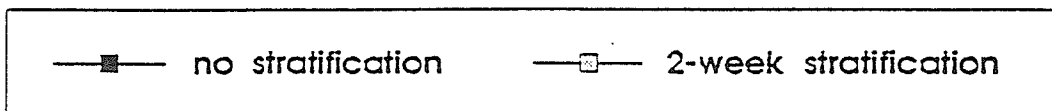
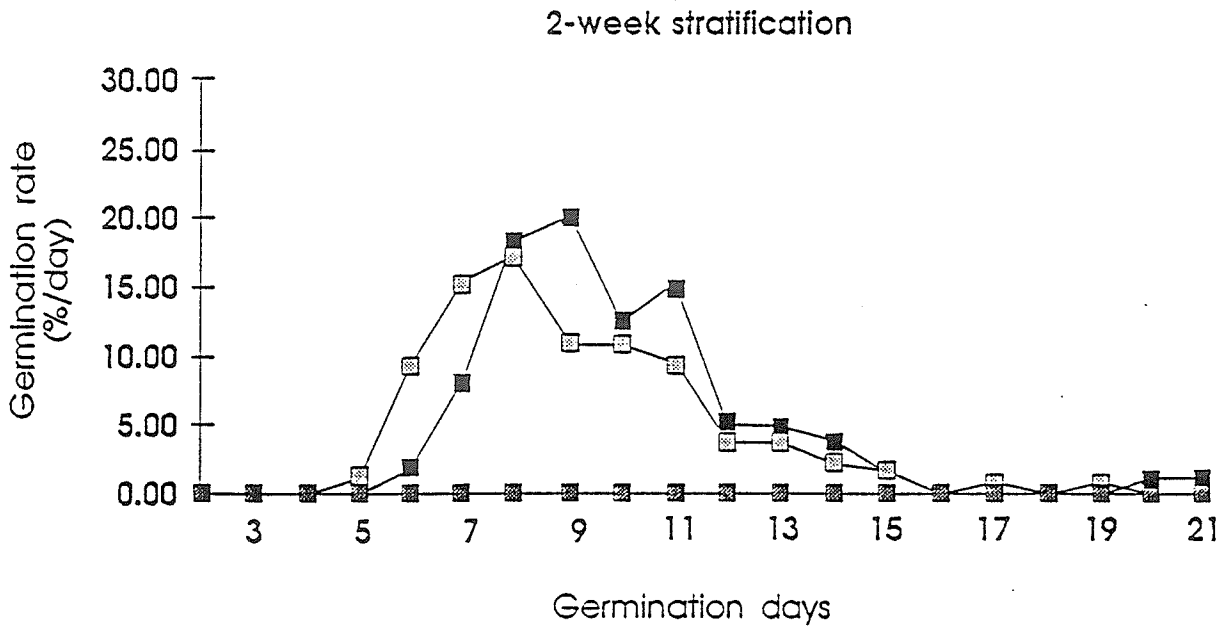
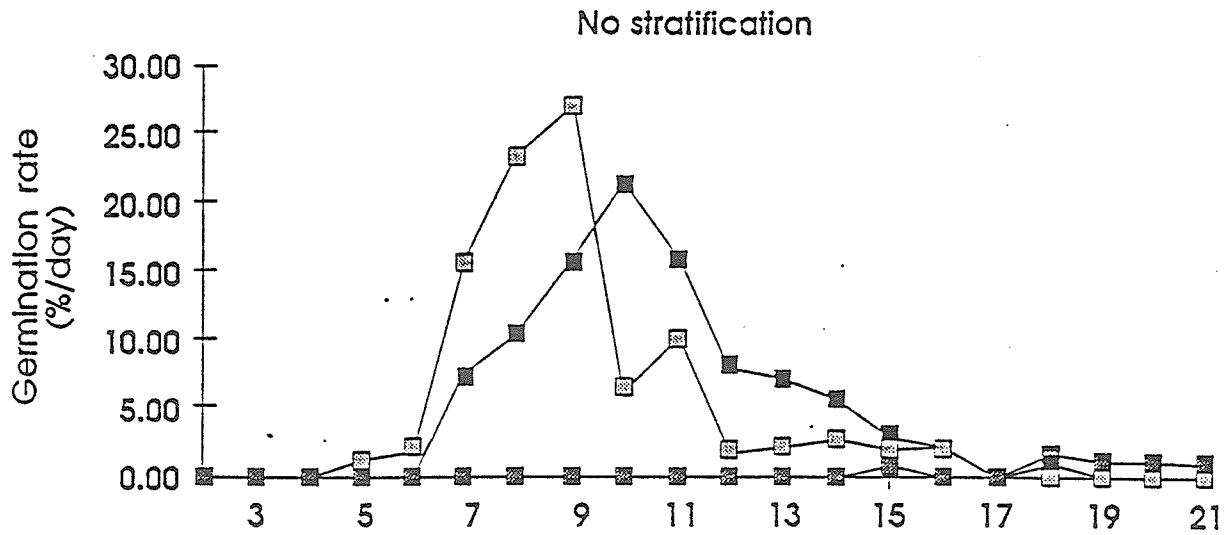


Figure 4

Figure 5. Effects of stratification treatment and incubation temperature on the germination of Dalbergia cochinchinensis seeds.

Dalbergia cochinchinensis

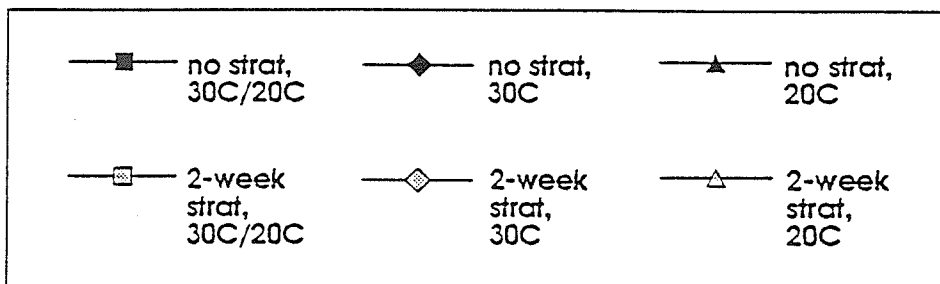
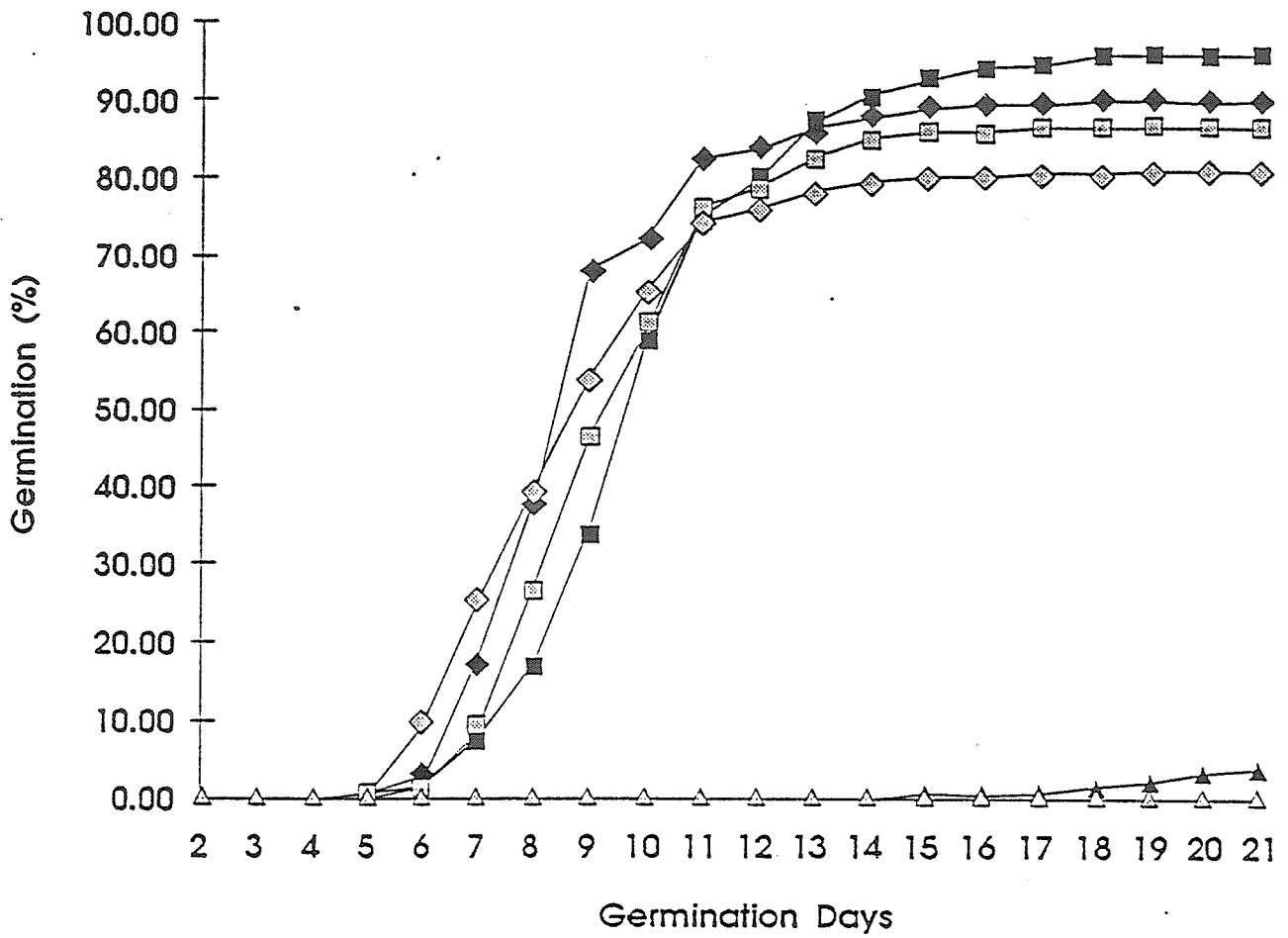


Figure 5

Figure 6. Effects of stratification on the germination rate of Pinus kesiya seeds at three incubation temperatures.

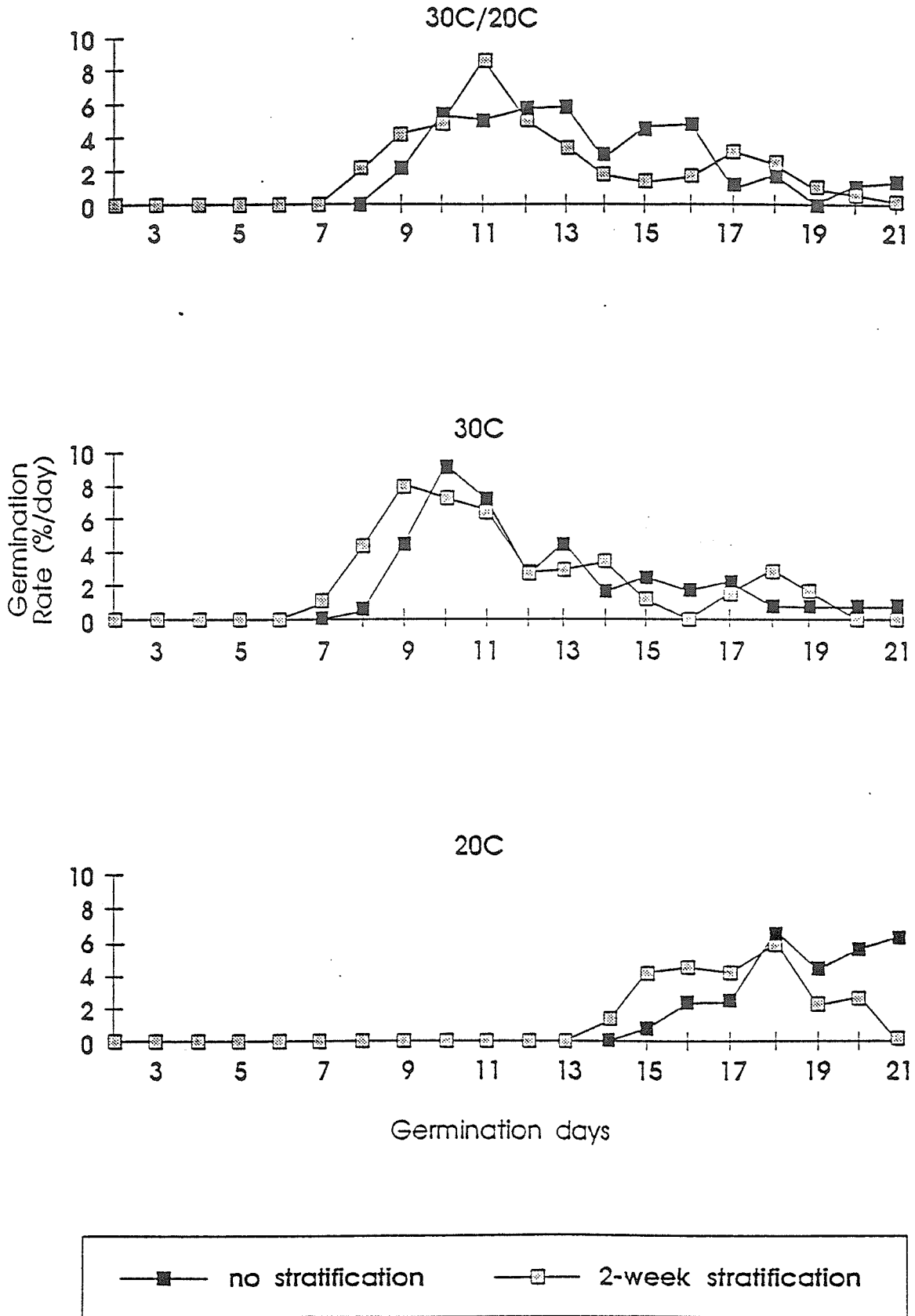


Figure 6

Figure 7. Effects of incubation temperature on the germination rate of Pinus kesiya seeds at two stratification levels.

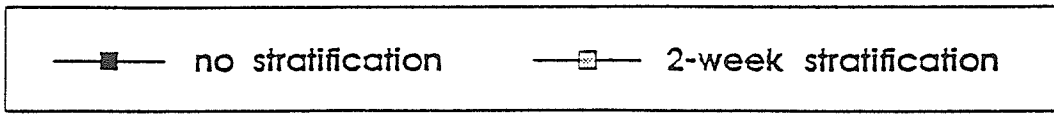
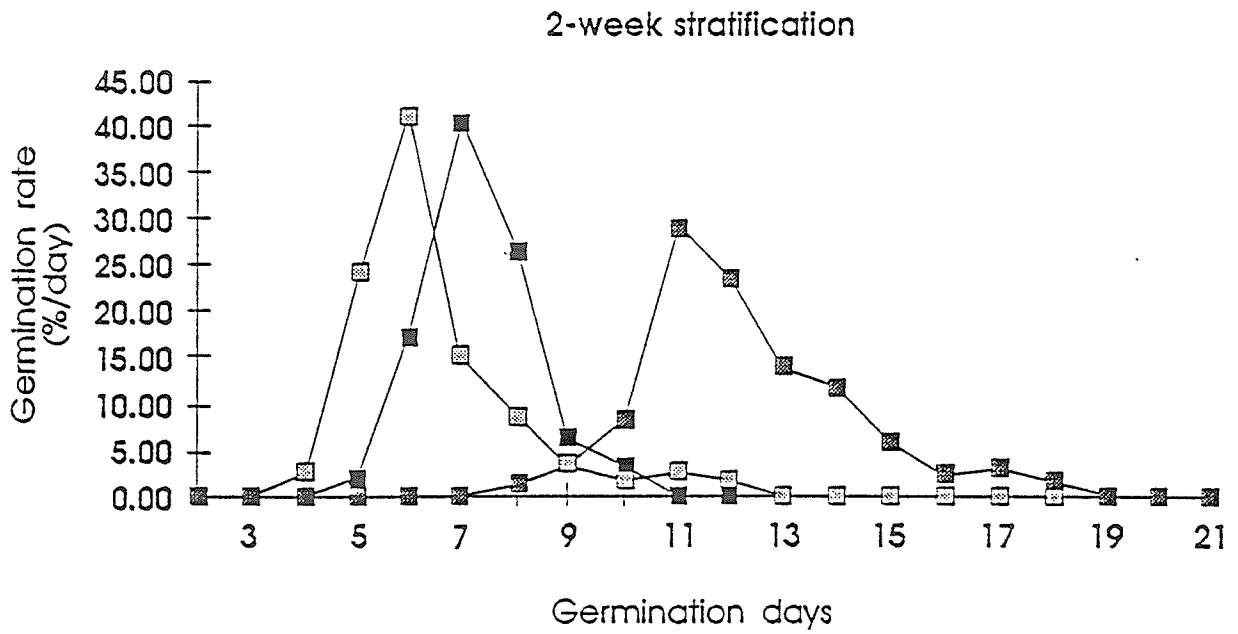
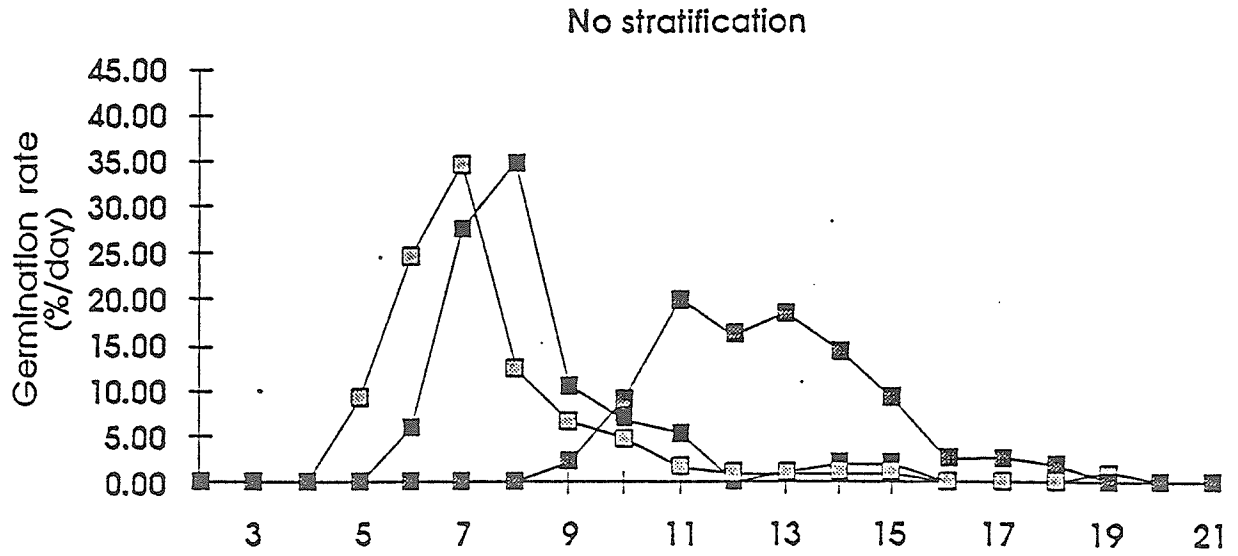


Figure 7

Figure 8. Effects of stratification treatment and incubation temperature on the germination of Pinus kesiya seeds.

Pinus kesiya

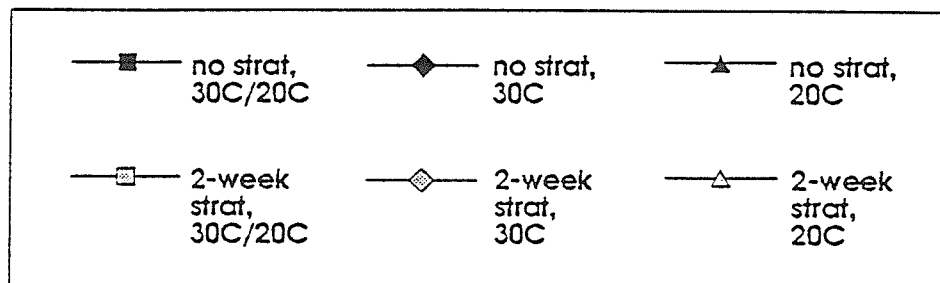
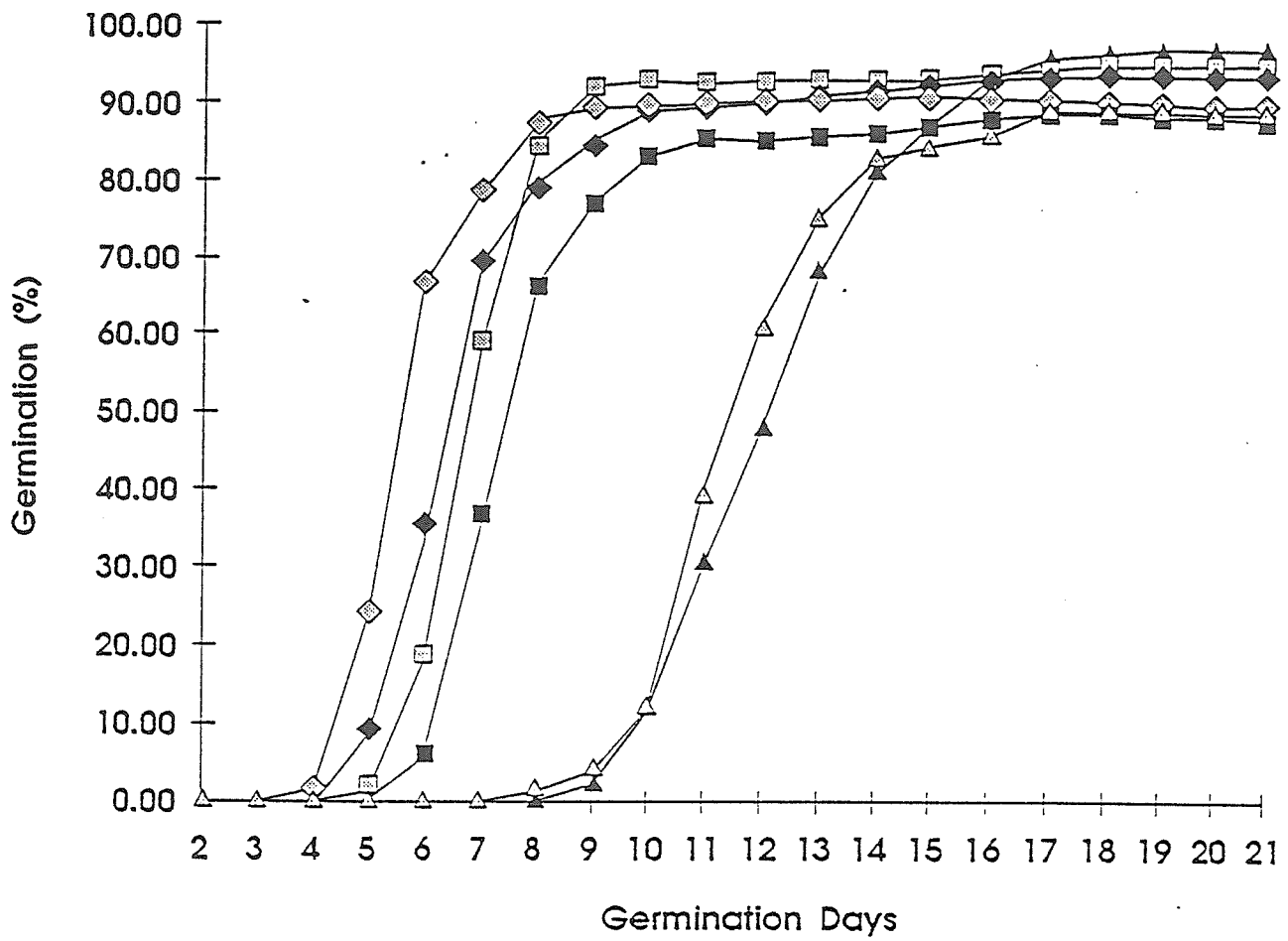
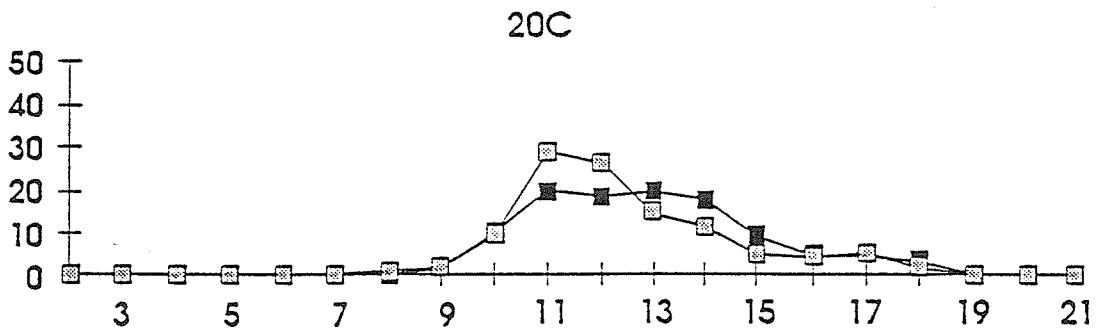
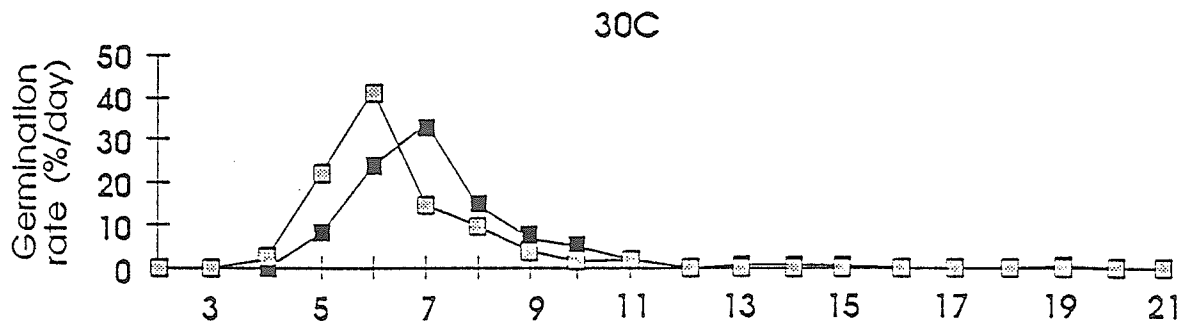
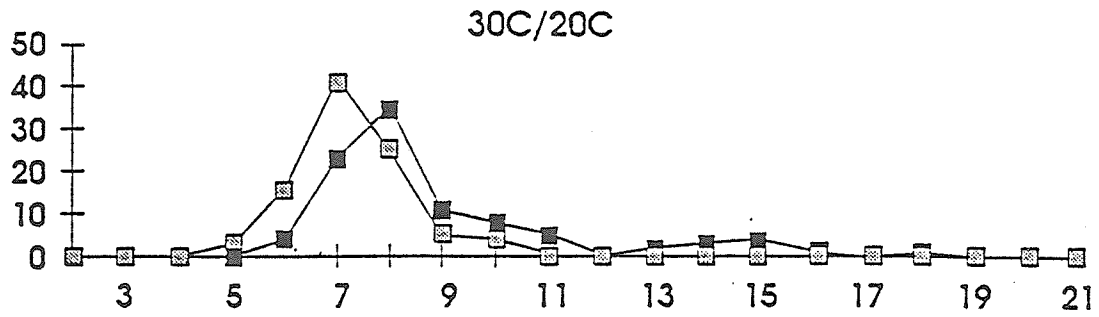


Figure 8

Figure 9. Effects of stratification on the germination rate of Pinus merkusii seeds at three incubation temperatures.



Germination days

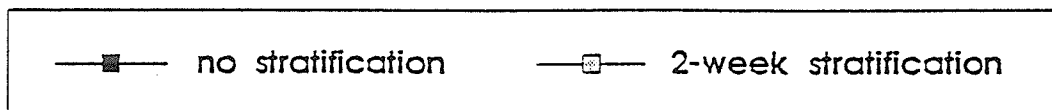


Figure 9

Figure 10. Effects of incubation temperature on the germination rate of Pinus merkusii seeds at two stratification levels.

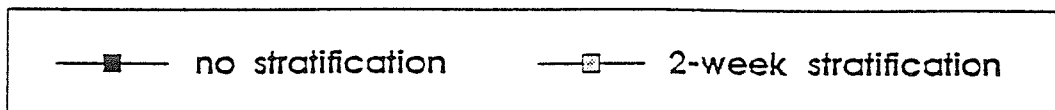
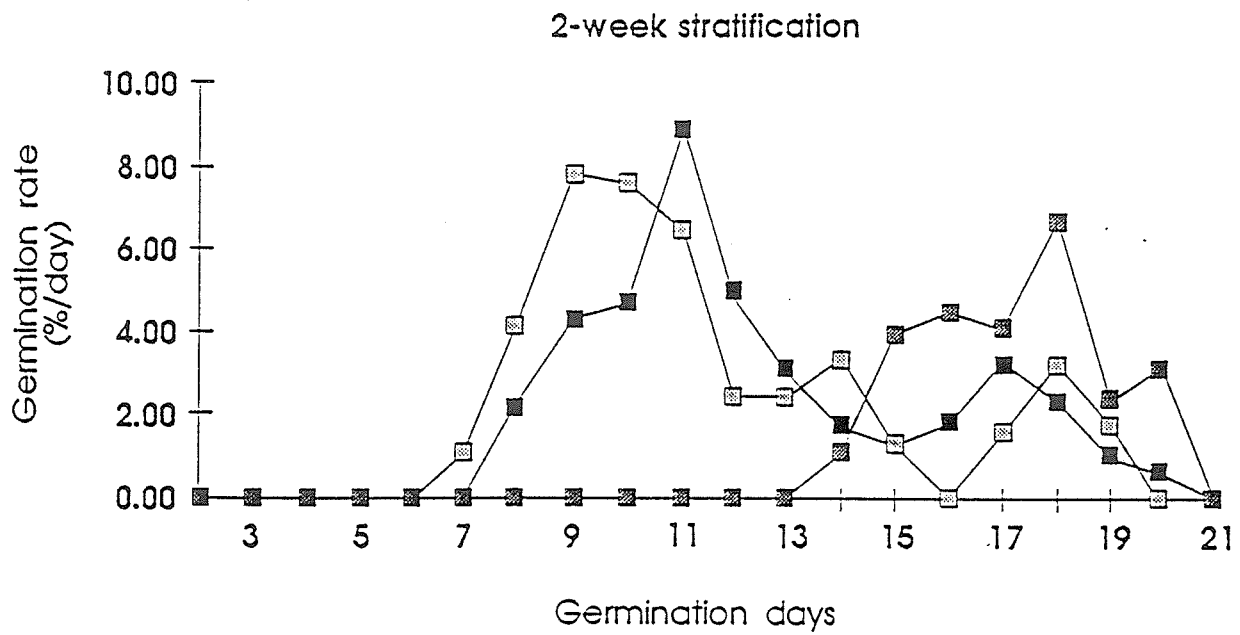
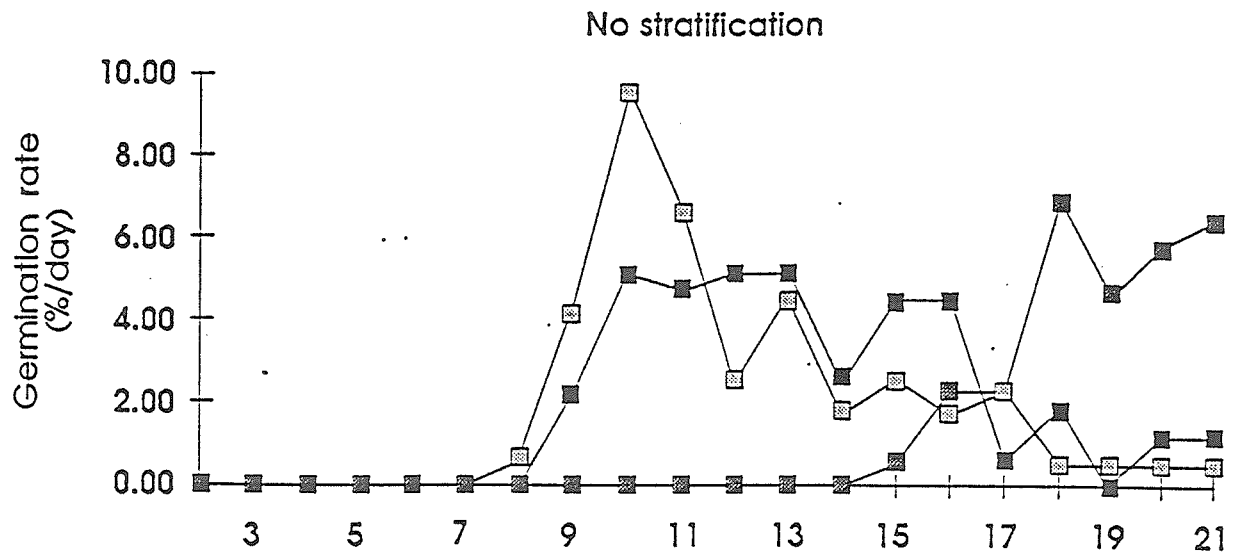


Figure 10

Figure 11. Effects of stratification treatment and incubation temperature on the germination of Pinus merkusii seeds.

Pinus merkusii

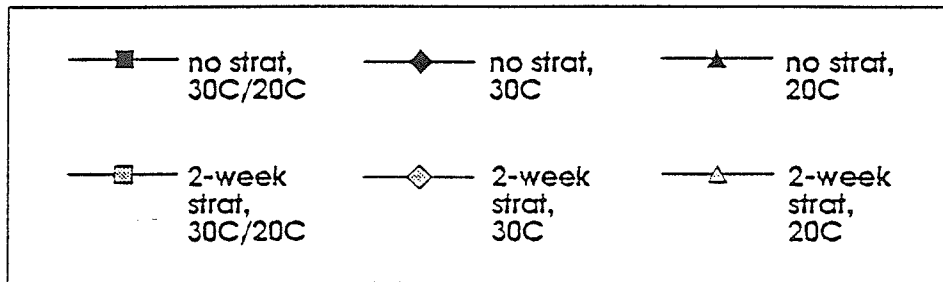
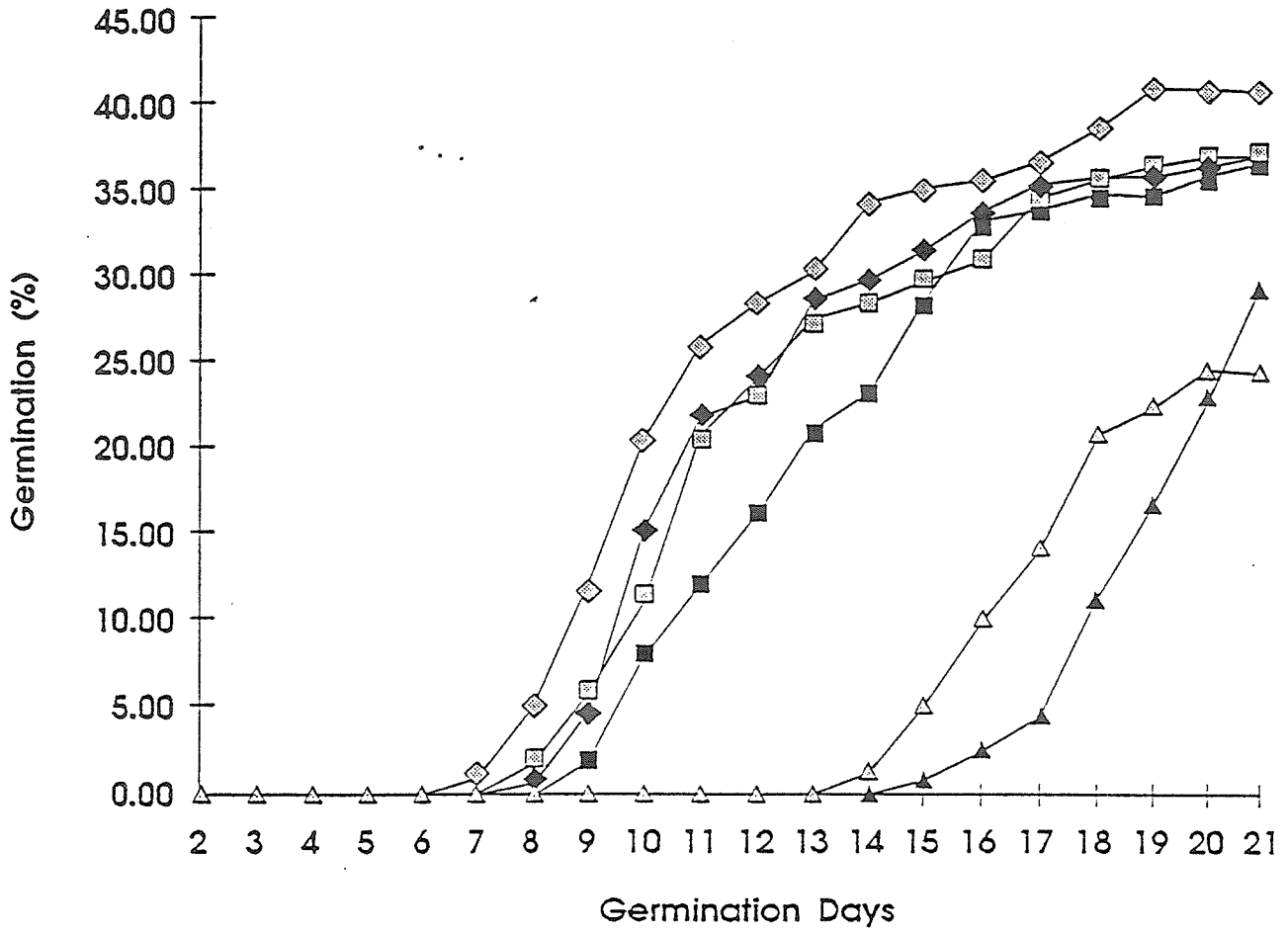


Figure 11