AN ABSTRACT OF THE THESIS OF

Marlene de Matos Malavasi for the degree of Doctor of Philosophy in

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Title: Physiological and Biochemical Studies of Stratified Douglas-fir [Pseudotsuga menziesii (Mirb.) Franco] Seeds After Redrying, Storage, and Subsequent Germination

Abstract approved: _____
Denis P. Lavender

The physiological and biochemical responses of Douglas-fir seeds to stratification, redrying, storage and subsequent germination were determined. Stratification increased seed vigor, embryo and gametophyte phosphorylative efficiency and RNA synthetic ability. Redrying seems to increase the rate of embryonic nucleic acid synthesis, speed of germination and seedling vigor. ATP, total adenosine phosphates and energy charge levels of stratified seed were stable through air drying. RNA levels were markedly reduced during 1 and 3 months of cold storage possibly indicating an enhanced RNAse activity. Storage also reduced seed energy status, seed viability, and seed vigor. The loss of stratification benefits and subsequent deterioration throughout storage were similar in stratified and redried stratified seeds. None of the biochemical criteria studied in the 5-day-old seedling showed close proportionality with either physiological responses or biochemical changes obtained in the seed stage.

Studies of water distribution among seed structures indicate that different tissues hydrate from an average of 6% to varied extent

after stratification. Stratified seeds contained 46, 56, 52 and 32% moisture content in whole seed, seed coat, embryo and gametophyte, respectively. Moisture reduction of stratified seeds to 35% did not affect embryo or gametophyte moisture content. However, redrying stratified seeds to 25% reduced the moisture content of all seed structures. Three months of storage did not alter moisture distribution within seeds.

Results suggest that it would be advantageous to redry seeds to a range of 25 to 35% before sowing to produce vigorous seedlings or to allow the expression of best benefits of stratification.

Physiological and Biochemical Studies of Stratified Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] Seeds After Redrying, Storage, and Subsequent Germination

by

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I dedicate this thesis to my supportive, loving family, Bira, Laís and Leticia. Without Bira's companionship, unending patience and understanding this thesis could not have been completed.

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CHAPTER I. INTRODUCTION

Delayed germination of Douglas-fir seeds results from the simultaneous effect of every seed component (Devillez, 1971). The seed coat slows down water and oxygen uptake, and embryo and gametophyte show a slow exchange between them. This process induces an embryonic dormancy which length depends on the provenance (Allen, 1960). Most chilling or stratification treatments give rise to a full embryo development and increase the inner exchange efficiency as also the water uptake through the seed coat.

Practical problems arise in connection with the storage of stratified seeds when unfavorable weather during the sowing season brings difficulty in matching the end of stratification with the desired sowing date. The preservation of surplus stratified seeds creates a related problem because losses of seeds by pregermination and deterioration may occur by lengthening the stratification period. There have been a few studies on moisture reduction and storage of stratified forest tree seeds. Barnett (1972) reported that stratified loblolly pine seeds (*Pinus taeda* L.) could be safely stored at 1°C for 12 months after drying to ten percent moisture content. Although total germination percent was not reduced, this procedure reinduced dormancy, necessitating restratification. Hedderwick (1968) air dried stratified Douglas-fir seeds for three weeks to an unknown moisture level and compared their germination with nondried stratified and

unstratified seeds. He found that air drying did not adversely affect seed viability. However, like Barnett, he found the benefits of stratification were lost and the seeds required stratification. In contrast, Allen (1962) found that even prolonged storage of Douglas-fir seeds following stratification and drying to about ten percent rarely offset the stratification effect completely and had little if any effect upon germination capacity in the case of high quality seed. Vanesse (1967) dried stratified Douglas-fir seeds to a moisture level below seven percent and found no adverse effect on seed viability. He also reported that these seeds could be stored at 5°C for "several weeks" before sowing. Further, Danielson and Tanaka (1978) stratified ponderosa pine (Pinus ponderosa Laws) and Douglas-fir seed lots, then dried these seeds to different moisture levels and stored at 2°C. They have found that air dried ponderosa pine seeds, with a moisture content of approximately 26 percent, could be stored for nine months without losing the beneficial effect of stratification or without their viability being adversely affected. Douglas-fir seeds showed a poorer response that was attributed to a higher seed moisture content (approximately 37 percent) during storage. Later, Edwards (1981) found that prechilled Abies seeds could be stored for a period up to 12 months at reduced moisture content without loosing the benefits of stratification.

The timing and sequence of events occurring during stratification are still not completely understood. Part of the research reported on the metabolic activity accompanying the afterripening of dormant tree seeds is focused on the synthetic ability of nucleic acid and protein, and phosphate metabolism.

Nucleotides and nucleic acids play an important role in protein synthesis, transmission of genetic information and response to environmental changes (Ching, 1972). Villiers (1972) suggested that a convenient control of dormancy would be the repression or derepression of the activities of the Deoxyribonucleic Acid (DNA), or an activation of the Ribonucleic Acid (RNA) in the process of protein synthesis because all metabolic activities of seeds depend on enzymes. However, only limited work has been done on the molecular level concerning the mechanism of stratification. Jarvis et al. (1968) have used gibberellin treatment substituting for stratification requirement in hazel (Corylus avellana L.) seeds. They found that the DNA template was doubled or tripled and rate of RNA polymerase activity was increased. Khan et al. (1968) showed that during stratification pear (Pyrus spp.) embryos developed increasing capacities for RNA synthesis as afterripening progressed and different kinds of RNA were synthesized. Tao and Khan (1974) demonstrated an increase in the activities of aminoacyl tRNA synthetases in pear embryos after as little as five (5) days of stratification. Further, Barnett and Adams (1974) in a study of the process of protein synthesis in sugar pine (Pinus lambertiana Dougl.) seeds at various times of stratification have reported that dry seeds contained only monoribosomes. The development of polyribosomes occurred during the first 36 hours of protein synthesis activity during stratification. Recently, Davies and Pinfield (1979) reported that in the embryonic axes of Norway maple (Acer platanoides L.) both the capacity for RNA synthesis and the levels of total RNA increased during stratification. Biological energy in the form of adenosine triphosphate (ATP) is needed in the synthesis of

proteins and nucleic acids, for transport, and movement. Therefore, ATP is in great demand during germination (Ching, 1972). Ching and Ching (1972) studying the germinating ponderosa pine seeds showed that after stratification the adenosine phosphate increased 7-fold and 6-fold in embryo and gametophyte, and energy charge rose to 0.85 and 0.75 respectively. The same researchers suggested that in Douglas-fir seeds, one of the mechanisms of dormancy appears to be the limitation of energy supply (Ching and Ching, 1973). Later, Szczotka and Tomaszewska (1980) and Murphy and Noland (1982) reported that ATP levels of Norway maple and sugar pine seeds increased during stratification. The present research was conducted to further study these biochemical effects of stratification on Douglas-fir seeds and also to investigate whether these effects are maintained or altered during air drying and storage and whether their maintenance or alteration is expressed once seeds have germinated. The physiological responses of Douglas-fir seeds to stratification, redrying storage and subsequent germination were monitored through germination percentage, speed of germination and seedling length and dry weight. In addition, because of the potentially important role of seed moisture content in the biochemical and physiological responses the distribution of water among seed parts was investigated in stratified and stratified redried seeds.

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CHAPTER II. PHYSIOLOGICAL RESPONSES OF DOUGLAS-FIR SEEDS TO STRATIFICATION, REDRYING, STORAGE AND SUBSEQUENT GERMINATION

Introduction

Moist chilling or stratification treatment is a commonly used technique for overcoming dormancy in seeds of many temperate species. Practical problems arise in connection with the storage of stratified seeds, when unfavorable weather during the sowing season brings difficulty in matching the end of stratification with the desired sowing date. In addition, the preservation of surplus stratified seeds creates a related problem because losses of seeds by pregermination and deterioration may occur by lengthening the stratification period.

There have been a few studies on moisture reduction and storage of stratified forest tree seeds. Barnett (1972) reported that stratified loblolly pine (Pinus taeda L.) seeds could be safely stored at 1°C for 12 months after drying to ten percent moisture content. Although total germination percent was not reduced, this procedure reinduced dormancy, necessitating restratification. Hedderwick (1968) air dried stratified Douglas-fir [Pseudotsuga menziesii (Mirb.) Franco] seeds for three weeks to an unknown moisture level and compared their germination with nondried stratified and unstratified seeds. He found that air drying did not adversely affect seed viability. However, like Barnett, he found the benefits of stratification were lost and the seeds required restratification. In contrast, Allen (1962) found that even prolonged storage of Douglas-fir seeds following stratification and drying to about ten percent rarely offset

the stratification effect completely and had little if any effect upon germinative capacity in the case of high quality seed. Vanesse (1967) dried stratified Douglas-fir seeds to a moisture level below seven percent and found no adverse effect on seed viability. He also reported that these seeds could be stored at 5°C for "several weeks" before sowing. Further, Danielson and Tanaka (1978) stratified ponderosa pine (Pinus ponderosa Laws) and Douglas-fir seed lots, then dried these seeds to different moisture levels and stored at 2°C. They have found that air dried ponderosa pine seeds, with a moisture content of approximately 26 percent, could be stored for nine months without losing the beneficial effect of stratification or without their viability being adversely affected. Douglas-fir seeds showed a poorer response that was attributed to a higher seed moisture content (approximately 37 percent) during storage. Later, Edwards (1981) found that prechilled Abies seeds stored for a period up to 12 months at reduced moisture content without loosing the benefits of stratification.

The present research was conducted to further study the physiological effects of stratification on Douglas-fir seeds and also, to investigate whether these effects are maintained or altered during air drying and storage and whether their maintenance or alteration is expressed once seeds have germinated.

Materials and Methods

Two Douglas-fir seed lots with high germinative capacity were used in this study. Seeds from the 1980 collection were obtained from a commercial seed company. One lot was collected at the coastal seed

zone 061 (elevation 0-500 ft) and the second at the interior seed zone 252 (elevation 501-1000 ft). Seeds were stored in airtight containers at 1°C until used. Before experimentation began seeds were screened to obtain large and uniform size. The seeds of both seed sources contained an average of 7% moisture.

Seeds were soaked in water at room temperature for 24 hr, drained, placed inside 4 mil polyethylene bags, then stratified at 3°C for 28 days. The moisture content of stratified seeds was adjusted to two lower levels: 25 percent and 35 percent. To reach the desired moisture content seeds were spread in a single layer over a mesh screen inside a standard room (temperature 21°C, RH 70 percent) for 20 minutes to 35 percent and 48 hours to 25 percent. Air dried seeds were placed in dry 4 mil polyethylene bags and returned to cold storage at 3°C for periods of 1 and 3 months. Non-dried, prechilled seeds were also stored. Their average moisture content was 45 percent for both seed lots.

Moisture contents were calculated after drying 4 samples of 10 seeds each for 24 hours at 105°C and expressed as a percentage of seed fresh weight by the formula:

$$\text{moisture content } \% = \frac{\text{fresh weight - dry weight}}{\text{fresh weight}} \times 100$$

To attain the target moisture contents, 10 samples of 10 seeds each from each lot were air dried inside the standard room for the following intervals: (1) every 5 minutes up to 1 hour, (2) every 1/2 hour up to 2 hours, (3) every hour up to 12 hours and (4) every 12 hours up to 48 hours. The mean moisture content was calculated by

the oven dry method as previously described. These means were used to decide the duration of the air drying process in order to reach the desired moisture contents (35 percent and 25 percent).

Germination was tested on 4 replications of 100 seeds each in clear, covered plastic dishes containing 200 ml of sterilized peat moss and vermiculite and 15 ml of water. Temperature alternated daily between 30°C for 8 hours and 20°C for 16 hours, with cool-white fluorescent lights (1000 lux) during the higher temperature period. Germinants were counted every second day, up to 28 days. Seeds were considered germinated when their radicles were at least 2 mm long.

Seed vigor was calculated on 4 replications of 100 seeds which were germinated as previously described. The vigor index was calculated by the following mathematical expression using germination speed as a measure of vigor:

$$\text{VIGOR INDEX} = \frac{\text{no. germinants (first count)}}{\text{no. of days to first count}} + \ldots$$

$$+ \frac{\text{no. germinants (last count)}}{\text{no. of days to last count}}$$

To obtain seedlings for length and dry weight measurements, which were performed 5 days after radicle emergence, four replications of 10 seedlings were taken from the germination test samples. Length measured in mm included the seedling extension from the tip of radicle to the top of cotyledons. Seedling dry weight was obtained by drying seedlings at 70°C until constant weight.

The various measurements were obtained by germinating dry seeds (7 percent moisture) taken direct from storage (nonstratified - NS) or after being submitted to one of the following treatments:

	Code
- Dry (nonstratified, nonstored control)	NS
- Soaked and stratified (stratified nonstored control)	S0
- Soaked, stratified and redried to 35 percent moisture content	S0D1
- Soaked, stratified and redried to 25 percent moisture content	S0D2
- Soaked, stratified and stored for 1 month (stratified stored control)	S1
- Soaked, stratified, redried to 35 percent moisture content and stored for 1 month	S1D1
- Soaked, stratified, redried to 25 percent moisture content and stored for 1 month	S1D2
- Soaked, stratified and stored for 3 months (stratified stored control)	S3
- Soaked, stratified, redried to 35 percent moisture content and stored for 3 months	S3D1
- Soaked, stratified, redried to 25 percent moisture content and stored for 3 months	S3D2

Statistical Analysis

Analysis of variance by complete randomized design was conducted on all data. T-tests were used to determine differences (1) among

diverse controls, and (2) between air drying treatments and controls in each storage period.

The following results compare averages in the following sequence: (1) stratified seed controls (45 percent m.c) of all storage periods, (2) stratified seed control (45 percent m.c) with stratified and air dried seeds (35 percent and 25 percent m.c) at each storage period and (3) nonstored stratified seed control with nonstratified seed control. For simplicity, occasionally the nonstratified control is referred to as NS and the stratified controls (45 percent m.c) at zero, one and three months of storage as S0, S1 and S3, respectively. The 5 percent probability level ($P < 0.05$) is used to test for statistical significance.

Results

Germination

The average cumulative germination percentages of seeds from zones 061 and 252 subjected to different treatments are summarized in Figures II.1 and II.5. It is clearly indicated by comparing the stratified controls S0, S1 and S3 that germination percentages in both sources are significantly reduced by 3 months of storage.

Generally, there were no significant differences in germination percent between control stratified seeds, 45 percent moisture content, and stratified seeds with moisture content adjusted to lower levels at all storage periods. The exceptions were: (1) Seeds from the coastal source with moisture content of 35 percent had a significantly lower germination capacity than the control after one month of storage, and

(2) The interior seeds with moisture content adjusted to 25 percent had a significantly higher germination percentage than control after 3 months of storage.

Figure II.5 shows that the average germination value of nonstratified control seeds (NS) from source 252 is significantly higher than the stratified control seed value (S0).

Seed Vigor Index

Storage progressively reduced seed vigor in the 061 source as is indicated by the significant differences among stratified controls S0, S1 and S3 (Fig. II.2). However, in seeds from the interior zone, seed vigor was reduced only by 3 months of storage (Fig. II.6).

The average vigor index became significantly larger than the control (S0) when nonstored stratified seeds from zone 061 had their moisture content reduced to 35 percent (S0D1). Seeds from zone 252 behaved similarly, however, the moisture reduction to 25 percent (S0D2) was also effective in increasing seed vigor. Generally, storage brought the altered vigor index down to the levels of the stored nondried stratified seeds (S0, S1, S3) with the exception of seeds from zone 252 which were air dried to 25 percent of moisture content and stored for 3 months (S3D2).

The average vigor index value of nonstored stratified control (S0) is significantly larger than that of nonstratified control (NS) in both seed sources (Figs. II.2 and II.6).

Seedling Length

Length of seedlings produced by control stratified seeds (45 percent m.c) of both 061 and 252 zones were gradually reduced throughout storage (Figures II.3 and II.7).

Seedlings originating from zone 061 seeds with moisture content 25 percent (S0D2, S1D2, S3D2) were significantly longer than controls at all storage periods. After 3 months of storage stratified seeds with 35 percent of moisture content (S3D1) also produced longer seedlings than the control (S3). Seeds from zone 252 had similar trends. However, nonstored stratified seed with 35 percent of moisture content (S0D1) also produced seedlings longer than the control (S0).

There were no differences in length of seedlings produced by nonstored stratified seeds (S0) and nonstratified seeds (NS) from either seed source.

Seedling Dry Weight

Figure II.4 indicates that stored stratified seeds (S1 and S3) from the coastal zone produced seedlings significantly lighter than nonstored stratified seeds(S0). In contrast, storage did not reduce the weight of seedlings produced by stored stratified seeds (S1, S3) from zone 252 (Figure II.8).

Seedling dry weight was significantly increased by reducing the moisture content of nonstored stratified seeds to 25 percent (S0D2). Seeds from both sources behaved similarly (Figures II.4 and II.8). The ability to produce heavier seedlings than the control (S1) was

preserved through on month of storage in seeds of zone 061. On the other hand, stratified seeds from the same source with moisture content reduced to 35 percent after being stored for 3 months (S3D1) produced seedlings lighter than the control (S3).

Seedling dry weight average value was not different for nonstored stratified seeds (S0) and nonstratified seeds (NS) from both 061 and 252 zones.

Discussion and Conclusions

Stratification caused a reduction in the germinative capacity of the interior seed lot. According to Lavender (1978) when seeds behave in this way it commonly indicates one or more of the following conditions: seeds were not fully mature when cones were harvested and extracted; seeds were damaged during processing; or seeds have deteriorated during storage. One or all these facts could possibly explain the reduction of percent of germination of seed lot 252, because we had no control over seed handling procedures before purchase, and we were unable to identify the precise cause.

Stratification indeed hastened germination speed in the seed lots. The present findings support those of Ching and Ching (1973). However, unlike our study, Ching and Ching found that stratification not only enhanced seed vigor but also resulted in significantly larger and heavier seedlings than the control. This disparity of results may be due to the fact that our seedling length and weight measurements were taken in an earlier developmental stage than those of Ching and Ching. Different seed lots and years may also affect the physiological responses.

Moisture reduction of stratified seeds to 35 and 25 percent remarkably increased seed vigor, seedling length and dry weight. Data suggest that seeds from both sources may have the best benefits of stratification with reduction of seed moisture to less than 35 percent. The physiological responses observed here are similar to the envigorating effect which increases seed performance and has been reported for grass, weed, crop and woody species (Hegarty 1978). In fact there are several claims in the literature that the use of presowing seed treatments involving the full or partial hydration of seeds, followed by subsquent dehydration, results in an increase in the rate, uniformity, and level of seed germination (Austin et al. 1969, Berrie et al. 1971, Hanson 1973).

The present findings suggest that metabolic processes induced by prechilling that often overcome seed dormancy and possibly initiated germination sensu strictu were merely arrested and not reversed by subsequent drying. There is no doubt that the air drying treatment had a substantial effect on the enhancement of seed germination and growth, however, the mechanisms of the effect are far from clear.

The studies reported in Chapter III indicate that the production of vigorous, heavy and large seedlings by stratified and redried seeds is accompanied by an increase in efficiency and/or activation for the synthesis of nucleic acids.

Further evidence for either accumulation of substrates or enhancement of activity of enzymes or systems in stratified and stratified and redried seeds is needed in order to explain the reason why the effect of physiological development on those seeds is not only preserved through partial dehydration but also enhanced.

Moist stratified seeds from both zones behaved similarly after storage at 2°C. There was a decrease in percent of germination, seedling vigor, seedling size and dry weight. The negative effects of storage were more dramatic in the coastal source. These results may be attributable to an early collection of cones. In Douglas-fir stratification beyond about 30 days was detrimental to total germination and seedling size of early collected seeds (Sorensen 1980).

Air dried stratified seeds didn't store better than those not air dried. Seeds from both zones with reduced moisture content had a significant decline in percent of germination, seed vigor and seedling length through storage. In addition, seedlings produced by coastal seeds at both reduced moisture levels had their dry weight reduced by storage.

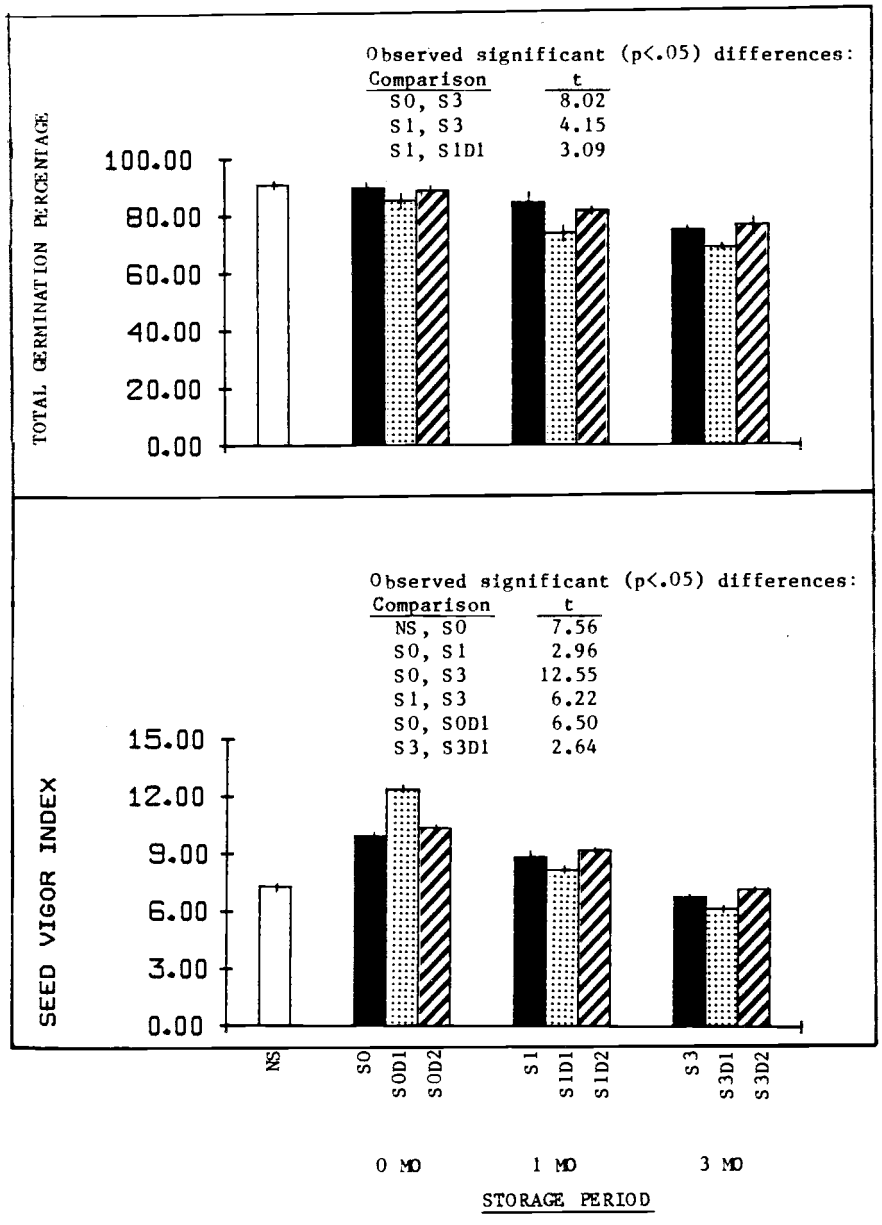
The reduction in final germination at all moisture content levels may be due to deterioration, rather than to the reinduction of dormancy, since most of the ungerminated seed were not viable.

These findings suggest that stratification benefits were gradually lost and deterioration began during storage regardless of seed moisture content.

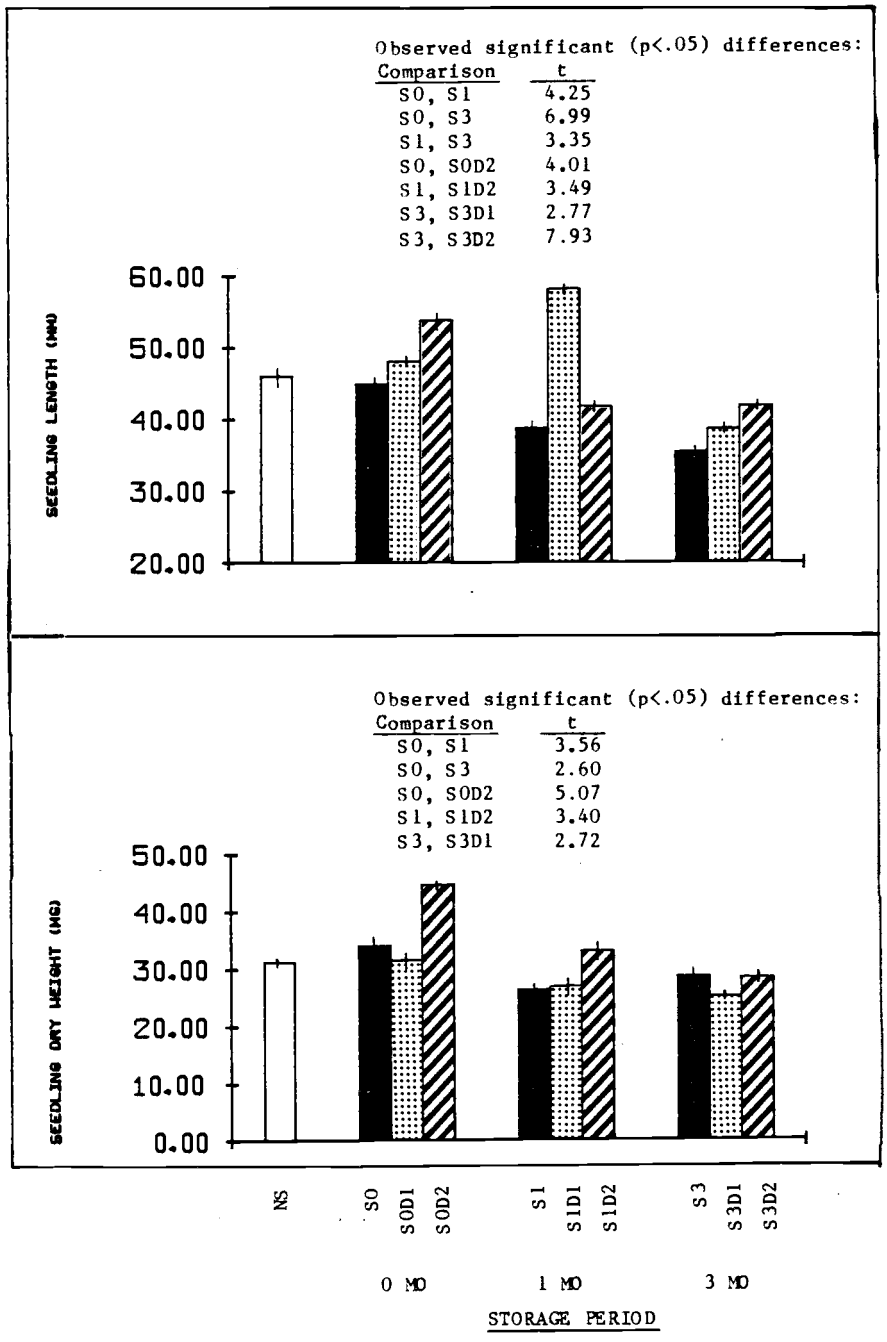
Danielson and Tanaka (1978) reported that the stratification effects, assessed by percent of germination and germination rate, were not lost when stratified Douglas-fir seeds were air dried and stored at 2°C. The increased germination rate over nonstratified seeds continued through 3 months storage. Similar studies done with ponderosa pine (Danielson and Tanaka 1978) and Abies (Edwards 1981) seeds revealed that not only can prechilled seeds be dried and stored for a considerable period, but significant increases in germination rate and

capacity can be obtained. Perhaps the varying responses between Danielson and Tanaka's Douglas-fir study results and ours can be attributed to the different seed sources and years used, since ecotypic variations in physiological behavior have been observed in Douglas-fir seeds from different provenances (Allen 1960). Another possible explanation for these physiological differences is that in addition to inherited potential for germination force and growth, prior history of seeds such as processing and storage methods may influence the degree of dormancy, and in turn seed response to environmental manipulations. Further, the possibility that different mechanisms control embryo dormancy in different species may account for the dissimilarity of results obtained in the ponderosa pine and Abies studies and ours.

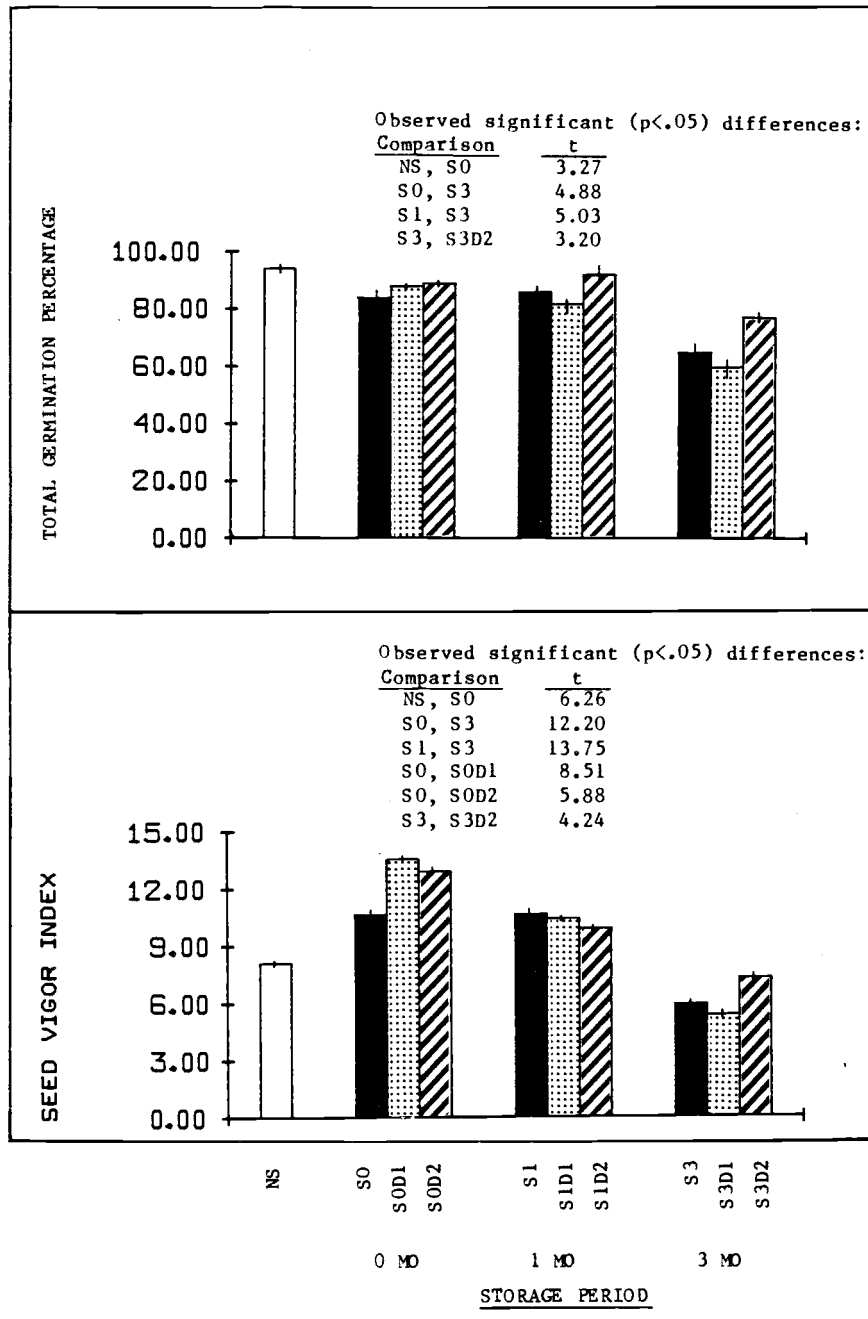
Based on the results of this study, we are skeptical about the advantages of reducing stratified seed moisture to preserve stratification benefits through long periods of storage. It appears that the air dried and non air dried stratified seeds from both 061 and 252 zones behaved similarly through storage. However, the striking increase in seed vigor, seedling length and dry weight achieved by reducing the moisture content of stratified seeds suggests that it would be advantageous to air dry stratified seeds before sowing. Because of seed zones' varied responses, the moisture reduction technique should be further investigated and pay special attention to provenances, maturity and processing procedures.



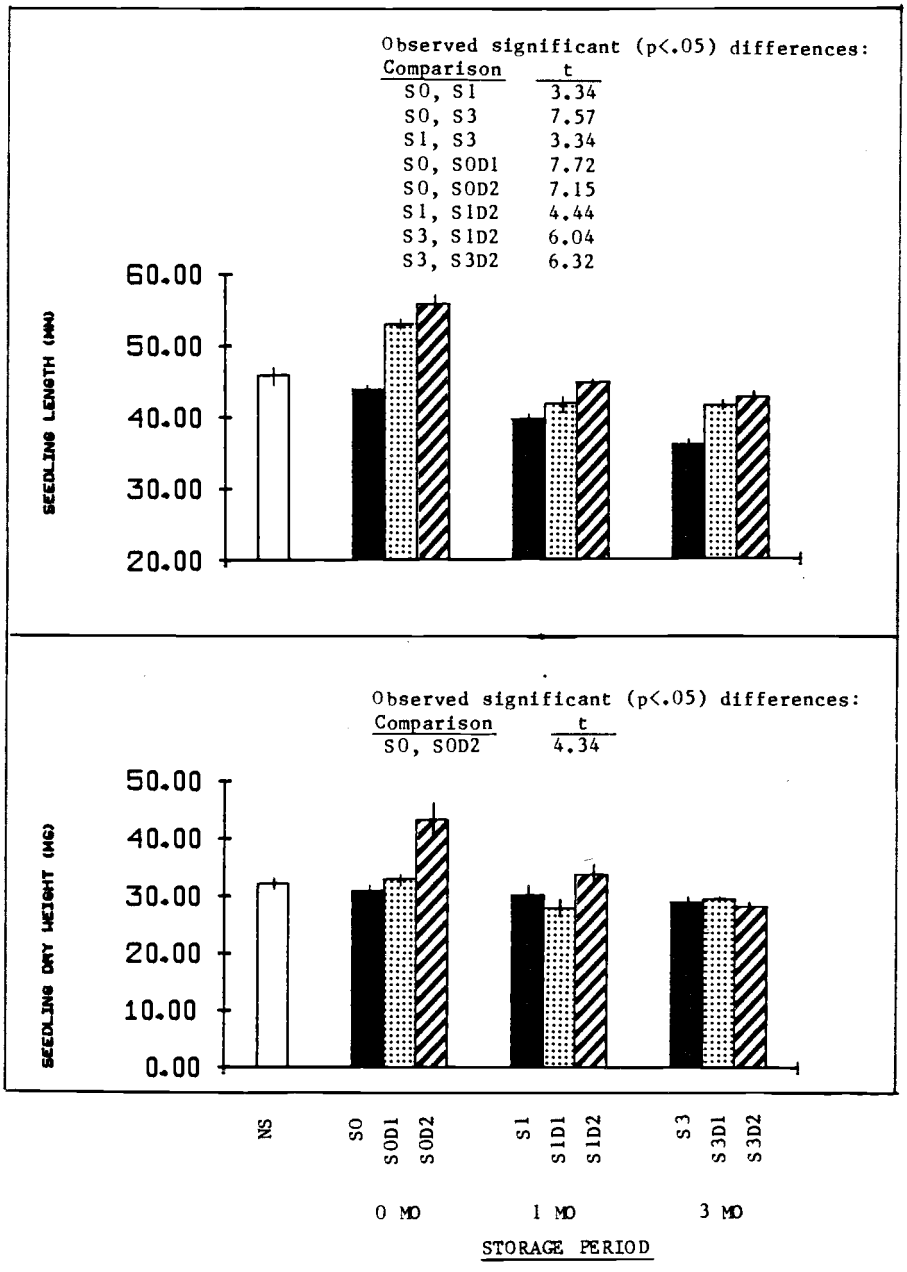
Figures II.1 and II.2. Effect of storage period and moisture content on germination percentage and seed vigor index of stratified Pseudotsuga menziesii seeds (seed zone 061). ■ - 45% moisture content, ⁞⁞ - 35% moisture content, ▨ - 25% moisture content. Bars represent, respectively, mean total germination and vigor index of 4 replications of 100 seeds. Error bars represent standard errors.



Figures II.3 and II.4. Effect of storage period and moisture content on 5-day-old seedling length and dry weight of stratified Pseudotsuga menziesii seeds (seed zone 061). ■ - 45% moisture content, ⁞⁞⁞ - 35% moisture content, ▨ - 25% moisture content. Bars represent, respectively, mean length and dry weight of 4 replications of 10 seedlings. Error bars represent standard errors.



Figures II.5 and II.6. Effect of storage period and moisture content on total germination percentage and seed vigor index of stratified Pseudotsuga menziesii seeds (seed zone 252). ■ - 45% moisture content, ⁞⁞ - 35% moisture content, ▨ - 25% moisture content. Bars represent, respectively, mean total germination and seed vigor index of 4 replications of 100 seeds. Error bars represent standard errors.



Figures II.7 and II.8. Effect of storage period and moisture content on 5-day-old seedling length and dry weight of stratified Pseudotsuga menziesii seeds (seed zone 252). ■ - 45% moisture content, ⁞⁞⁞ - 35% moisture content, ▨ - 25% moisture content. Bars represent, respectively, mean length and dry weight of 4 replications of 10 seedlings. Error bars represent standard errors.

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CHAPTER III. BIOCHEMICAL RESPONSES OF DOUGLAS-FIR SEEDS TO STRATIFICATION, REDRYING, STORAGE AND SUBSEQUENT GERMINATION

Introduction

Stratification also known as prechilling has long been used to hasten dormant seed germination of temperate zone species and has become common practice in tree nurseries of the Pacific Coast. Thus, two of the commonest problems in nursery management are to match the end of stratification with the desired sowing date and the preservation of surplus stratified seed because seed losses often occur if the optimum length of stratification is exceeded.

A few reports have indicated that stratified seeds of Douglas-fir [Pseudotsuga menziesii (Mirb.) Franco] (Vanesse 1967, Hedderwick 1968) and loblolly pine (Pinus taeda L.) (Barnett 1972) may be dried to approximately 10% moisture content and stored at low temperature without losing viability. However, the effect of prechilling treatment was lost.

Recently, Danielson and Tanaka (1978) reported that stratified ponderosa pine seeds (Pinus ponderosa Laws) after being redried to approximately 26% moisture content were stored for nine months without losing the beneficial effect of stratification or without their viability being adversely affected. Douglas-fir seeds showed a poorer response which was attributed to a higher moisture content during storage (approximately 37 percent).

Later, Edwards (1981) found that stratified Abies seeds that had been air dried to approximately 25% moisture content were successfully

stored for 12 months without reduction in either viability or stratification effect. This study also indicated that air drying stratified seeds stimulated germination to much higher levels than achieved by stratification alone. In addition, results reported in Chapter II indicated that the reduction of moisture content in stratified Douglas-fir seeds to 25 or 35% promoted the production of more vigorous, heavier and larger seedlings than stratification alone.

Although some work has been done on the physiology of redrying stratified seeds, there is little information about metabolic changes occurring during the process.

The present study was initiated in an attempt to investigate whether some of the known biochemical effects of stratification are maintained or altered during redrying and storage and whether their maintenance or alteration is expressed once seeds have germinated.

The biochemical effects of stratification studied were: quantitative changes in total nucleotides, nucleic acids and adenosine phosphates. These biochemical events were selected because there are several claims in the literature that the breakage of seed dormancy by processes such as stratification may be accompanied by an increase in synthetic capacity of nucleotides and nucleic acids (Wood and Bradbeer 1967, Jarvis, Frankland and Cherry 1968, Khan, Heit and Lippold 1968, Villiers 1972, Tao and Khan 1974, Davies and Pinfield 1979) and by an elevation of energy status (Ching and Ching 1972, 1973, Szczotka and Tomaszewska 1980, Murphy and Noland 1982).

Materials and Methods

Two Douglas-fir seed lots with high germinative capacity were used in this study. Seeds from the 1980 collection were obtained from a commercial seed company. One lot was collected in the coastal seed zone 061 (elevation 0-500 ft) and the second in the interior seed zone 252 (elevation 501-1000 ft). Seeds were stored in airtight containers at 1°C until used. Before experimentation began seeds were screened to obtain large and uniform size. Seed of both sources contained an average of 7% moisture.

Seeds were soaked in water at room temperature for 24 hr, drained, placed inside 4 mil polyethylene bags, then stratified at 3°C for 28 days. The moisture content of stratified seeds was adjusted to two lower levels: 25 percent and 35 percent. To reach the desired moisture content seeds were spread in a single layer over a mesh screen inside a standard room (temperature 21°C, RH 70 percent) for 20 minutes to 35 percent and 48 hours to 25 percent. Air dried seeds were placed in dry 4 mil polyethylene bags and returned to cold storage at 3°C for periods of 1 and 3 months. Non-dried, prechilled seeds were also stored. Their average moisture content was 45 percent for both seed lots.

Moisture contents were calculated after drying 4 samples of 10 seeds each for 24 hours at 105°C and expressed as a percentage of seed fresh weight by the formula:

$$\text{moisture content } \% = \frac{\text{fresh weight - dry weight}}{\text{fresh weight}} \times 100$$

To attain the target moisture contents, 10 samples of 10 seeds each from each lot were air dried inside the standard room for the following intervals: (1) every 5 minutes up to 1 hour, (2) every 1/2 hour up to 2 hours, (3) every hour up to 12 hours and (4) every 12 hours up to 48 hours. The mean moisture content was calculated by the oven dry method as previously described. These means were used to decide the duration of the air drying process in order to reach the desired moisture contents (35 percent and 25 percent).

The various biochemical determinations were performed on seeds or seedlings from each of the following treatments

	Code
- Dry (nonstratified, nonstored control)	- NS
- Soaked and stratified (stratified nonstored control)	- S_0
- Soaked, stratified and redried to 35 percent moisture content	-S_0D_1
- Soaked, stratified and redried to 25 percent moisture content	-S_0D_2
- Soaked, stratified and stored for 1 month (stratified stored control)	- S_1
- Soaked, stratified, redried to 35 percent moisture content and stored for 1 month	-S_1D_1
- Soaked, stratified, redried to 25 percent moisture content and stored for 1 month	-S_1D_2

- Soaked, stratified and stored for 3 months (stratified stored control) - S_3
- Soaked, stratified, redried to 35 percent moisture content and stored for 3 months -S_3D_1
- Soaked, stratified, redried to 25 percent moisture content and stored for 3 months -S_3D_2

Adenosine phosphates, nucleotides and nucleic acids determinations were performed at following germination developmental stages: (1) 0-day germination (embryo axis and gametophyte), (2) Five days after radicle emergence (seedling). Seeds were germinated in clear, covered plastic dishes containing 200 ml of sterilized peat moss and vermiculite and 15 ml of water. Temperature alternated daily between 30°C for 8 hours and 20°C for 16 hours, with cool-white fluorescent lights (1000 lux) during the higher temperature period.

Extraction of adenosine phosphates, nucleotides and nucleic acids. - Three replications of 20 seeds were dissected to gametophytes and embryos on chilled moist filter paper. Dry seeds were dissected on dry filter paper. Gametophytes, embryos and three replications of four 5-day-old seedlings were used for the extraction of adenosine phosphates, nucleotides, and nucleic acids. Extraction was done by grinding the embryos, or gametophytes or seedlings with 0.25 M perchloric acid ($HClO_4$) (5 ml for embryos, 10 ml for seedlings and gametophytes) in a chilled porcelain mortar with pestle to a smooth paste (Ching 1981). The slurry extract was centrifuged at 10 kg for 10 minutes. The precipitate was stored for nucleic acids analysis. The supernatant was neutralized with 2 N KOH (.6 ml for embryos, 1.2

ml for gametophytes and seedlings) and 0.1 M N-2-hydroxyethyl piperazine-N'-2-ethanesulfonic acid (HEPES) buffer, pH 7.0 (1 ml) and titrated with KOH to pH 7± 0.1. The neutralized extract was centrifuged at 10 Kg for 5 minutes and the precipitate removed. An aliquot of the neutralized extract was used for adenosine phospates assay and the remaining solution for nucleotides estimation.

Estimation of adenosine phosphates and adenylate energy charge

Adenosine phosphate levels were estimated by the luciferin-luciferase method using an Aminco Chem-Glow photometer (Ching and Ching 1972). Freeze-dried firefly extract containing luciferin-luciferase was purchased from the Sigma Chemical Co.,.St. Louis, Mo. (FLE-50). The neutralized extract was properly diluted to the instrument sensitivity with 0.025M HEPES buffer containing 0.025M Mg acetate, pH 7.5. Adenosine triphosphate (ATP) was determined in the diluted extract. Adenosine diphosphate (ADP) was converted to ATP by phosphoenol-pyruvate and pyruvate kinase (EC 2.7.1.40) and then assayed. Adenosine monophosphate (AMP) was converted to ADP with endogenous ATP by adenylate kinase (EC 2.7.4.3) and the resulting ADP was converted to ATP and assayed. Based on the integrated area under the curve of light intensity produced from the three reaction mixtures, ATP concentration were read directly from a standard curve of freshly prepared ATP solution and the particular batch of enzyme-substrate prepared for the assay. Adenylate Energy Charge (EC) was calculated according to Atkinson (1969); EC = ([ATP] + 1/2[ADP])/([ATP]+[ADP]+[AMP]).

Estimation of acid soluble nucleotides and nucleic acids

Nucleotides in the neutralized extract were separated by ion-exchange chromatography using a Dowex 1-x8 resin, 50-100 mesh column and estimated from the A260 of the ammonium formate eluate (Ching 1966).

Ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) were assayed by the diphenylamine and orcinol procedure in the $HClO_4$-extracted residue (Ching 1966). Highly purified yeast RNA and highly polymerized calf thymus DNA (all from Sigma) were carried through the procedures, and were used as standards for quantitative estimation.

Statistical Analysis

Analysis of variance by complete randomized design was conducted on all data. T-tests were used to determine differences (1) among diverse controls, and (2) between air drying treatments and controls in each storage period.

The following results compare averages in the following sequence: (1) nonstratified seed control (NS) with stratified nonstored seed control (S0), (2) stratified seed controls of all storage periods (S0, S1, S3) and (3) stratified seed controls (S0, S1, S3) with stratified and redried seeds at each storage period (S0D1, S0D2, S1D1, S1D2, S3D1, S3D2). The 5 percent probability level ($P<0.05$) is used to test for statistical significance.

Results

Embryo

A. Energy status

1. Effects of stratification

The average nanomole of ATP for embryos of sources 061 and 252 are shown in figures III.1 and III.2, respectively. Stratification significantly increased ATP levels as it is indicated by the differences between nonstratified (NS) and stratified (S0) control means. A similar trend was observed in embryos of both seed sources.

Stratification significantly reduced the ADP level of embryos from source 061 and did not affect AMP levels (Figs. III.3 and III.5). In contrast, embryos of interior seeds had their ADP levels increased and AMP level reduced during stratification (Figs. III.4 and III.6). Total adenosine phosphates (TAP) levels of stratified seed embryos (S0) were significantly larger than nonstratified seeds (NS) in both seed sources (Figs. III.7 and III.8).

During stratification adenylate energy charge of embryos significantly rose from values around .5 in nonstratified seeds (NS) to about .8 in stratified control (S0). Seeds from both zones behaved similarly (Figs. III.9 and III.10).

2. Effects of storage following stratification

The average ATP levels of stratified seed embryos from zone 061 became significantly smaller than the control (S0) after 3 months of

storage (S3) (Fig. III.1). Storage didn't cause significant reduction in ATP level in embryos of zone 252 (Fig. III.2).

ADP levels of stratified coastal seed embryos after being stored for one month (S1) were significantly higher than the nonstored stratified control (S0) (Fig. III.3). However, there was no observable difference between embryo ADP level of stratified nonstored (S0) and stratified and stored for 3 months seeds (S3). ADP level of embryos of the interior zone was reduced after 3 months of storage (Fig. III.4).

Embryo AMP levels in the coastal seeds increased markedly after one month of storage (S1) and remained at the same level through 3 months of storage (S3) (Figure III.5). On the other hand, Figure III.6 indicates that embryo AMP level of interior stratified seeds gradually increased during the three months of storage (S1 and S3).

In source 061, embryo TAP levels increased after one month of storage (Fig. III.7). In the interior source, embryo TAP level remained constant (Fig. III.8). Prolonged storage (3 mo) significantly decreased TAP level in the coastal stratified seeds (S3) (Fig. III.7).

Storage significantly reduced EC in stratified seed embryos from zone 061, while in the interior seeds the EC remained unchanged (Figs. III.9 and III.10).

3. Effects of moisture reduction and storage following stratification

There were no significant differences between embryo ATP levels of stratified controls (S0, S1, S3) and stratified and redried seeds (S0D1, S0D2, S1D1, S1D2, S3D1, S3D2) at all storage periods (Figs.

III.1 and III.2). Seeds from both 061 and 252 sources behaved similarly.

Embryos of stratified and redried seeds (S0D1, S0D2) from zone 061 had a significantly higher ADP level than the stratified nonstored control (S0) (Fig. III.3). After 1 month of storage these embryos (S1D1, S1D2) had lower ADP levels than the stratified stored control (S1). However, after 3 months of storage the ADP level of stratified and redried seed embryos (S3D1, S3D2) were again higher than the stratified and stored control (S3). Redrying significantly reduced ADP levels in embryos of source 252 (S0D1, S0D2). However, under storage conditions there were no differences between ADP levels of stratified and redried seed embryos (S1D1, S1D2, S3D1, S3D2) and controls (S1, S3) (Fig. III.4).

In embryos of zone 061, there were no differences in AMP levels between stratified and redried treatments (S0D1, S0D2) and the stratified nonstored control (S0) (Fig. III.5). One month of storage resulted in greater increase in AMP levels of stratified seed embryos (S1) than in stratified and redried seed embryos (S1D1, S1D2) (Fig. III.5). After 3 month storage AMP levels of S3D2 were still increasing, while those of S3 and S3D2 had fallen below one-month levels. At this point there was no significant difference between S3D2 and S3, while S3D1 was less than both. Interior seeds followed a different pattern (Fig. III.6). Embryos of stratified and redried seeds (S0D1, S0D2) had a significantly higher AMP level than the control (S0). However, after 3 months of storage embryo AMP level of stratified and redried to 35% moisture seeds (S3D1) was lower than the control (S3).

Generally there was no significant difference between embryo TAP levels of stratified and redried seeds (S0D1, S0D2, S1D1, S1D2, S3D1, S3D2) and the controls (S0, S1, S3) at all storage periods in both sources (Figs. III.7 and III.8). The exception was a significant decrease in TAP level in the embryos of stratified and redried to 25% moisture coastal seed after one month of storage.

Redrying without storage didn't effect energy charge in either seed source, however, in source 061 it reduced loss in energy charge with storage, especially for S1D1. Thus, after one month, energy charge of S1 was less than both S1D1 and S1D2, and after 3 months S3 was still less than S3D1 (Fig. III.9). Zone 252 seed followed somewhat the same pattern, however, differences between dried and non-dried seed were not significant (Fig. III.10).

B. Nucleic acids and nucleotides

1. Effects of stratification

RNA levels of nonstratified (NS) and stratified seed embryos (S0) were different. Stratification raised embryo RNA levels, however there were no observable difference in the levels of DNA and nucleotides. Seeds from both 061 and 252 sources had similar trend (Figs. III.11 to III.16).

2. Effects of storage following stratification.

Storage markedly reduced embryo RNA levels of stratified seeds (S0, S1 and S3; Figs. III.11 and III.12). In contrast, storage did

not alter embryo DNA and nucleotides levels (Figs. III.13-16). Embryos of both 061 and 252 had similar trends.

3. Effects of moisture reduction and storage following stratification

In source 061, embryo RNA levels of stratified and redried seeds (S0D1, S0D2) were significantly higher than the control S0 (Figure III.11). Storage reduced RNA levels in dried and non-dried seed from both sources. After 3 months embryo RNA levels of stratified and redried to 25% moisture seeds (S3D2) were higher than control S3. Generally, in source 252 embryo RNA levels of stratified and redried seeds (S0D1, S0D2, S1D1, S1D2, S3D1, S3D2) did not significantly differ from controls S0, S1 and S3 (Fig. III.12). However, a trend similar to the source 061 data can be observed. One exception is that after one month of storage RNA levels of stratified and redried seeds are significantly higher than the control S1.

Moisture reduction significantly increased embryos DNA levels of stratified seeds (S0D1, SA0D2) from zone 061 (Fig. III.13). The higher DNA levels were preserved through 1 month of storage in stratified and redried to 25% moisture seeds (S1D2). After 3 months of storage the DNA levels of stratified and redried to 35% moisture seeds were markedly lower than the control S3. In the interior source, embryo DNA levels of stratified and redried to 35% moisture seeds (S0D1) were also higher than the control S0 (Fig. III.14). Otherwise there were not statistically significant differences between stratified and redried seeds (S0D2, S1D1, S1D2, S3D1, S3D2) and their

controls S0, S1 and S3. However, these data follow a pattern similar to source 061.

Moisture reduction to 35% significantly increased embryo nucleotide levels in stratified coastal seeds (S0D1) (Fig. III.15). After one month of storage the embryos of stratified and redried to 25% moisture seeds (S1D2) had higher nucleotide levels than the control S1. In zone 252 average nucleotides of S0D1 were also greater than those of S0, however, the difference wasn't significant at the .05 level. In general there were no significant differences in embryo nucleotide levels between stratified and redried seeds (S0D1, S0D2, S1D1, S1D2, S3D2) and controls (S0, S1 and S3) (Fig. III.16). However after 3 months of storage embryos of stratified redried to 25% moisture (S3D2) had higher nucleotide levels than the control S3.

Gametophyte

A. Energy status

1. Effect of stratification

The average ATP and AMP for gametophytes of sources 061 and 252 are illustrated in Figs. III.17, III.21, and III.18, III.22 respectively. Stratification significantly changed the levels of these two adenylate species. There were a marked increase in ATP levels in both sources and a decrease of AMP in coastal source.

ADP levels of gametophytes didn't differ between stratified (S0) and nonstratified seeds (NS) in either source (Figs. III.19 and III.20).

Stratification significantly increased gametophyte TAP levels in both sources (Figs. III.23 and III.24).

Gametophyte energy change markedly rose from .34 to .76 in coastal seeds and from .54 to .77 in interior seeds (Figs. III.25 and III.26).

2. Effects of storage following stratification

One month of storage markedly increased ATP, ADP and AMP levels of gametophytes from source 061 (S1) (Figs. III.17, III.19 and III.21). In contrast, after 3 months of storage ATP and AMP levels were significantly lower than the control SO. Gametophytes of interior seeds exhibited a different trend under storage conditions. Three months of storage significantly reduced ATP and increased AMP levels (Figs. III.18 and III.22), otherwise there were no difference in adenosine phosphate levels between stratified and stored seeds (S1, S3) and the SO control.

In stratified coastal seeds, gametophytes TAP levels increased during one month of storage (S1), but prolonged storage (3 month) significantly reduced the adenylate pool (Fig. III.23). There were no differences in gametophyte TAP levels between stratified and stored for one month interior seeds (S1) and the control SO (Fig. III.24). However, as in the coastal source, gametophyte TAP levels were markedly reduced by three months of storage.

Gametophyte energy charge was not significantly altered during storage in either source (Figures III.25 and III.26).

3. Effects of moisture reduction and storage following stratification

Moisture reduction markedly changed gametophyte ATP levels in stratified seeds from zone 061 (Fig. III.17). ATP levels of stratified and redried seeds (S0D1, S0D2) were significantly higher than the control S0. One month storage reduced the altered ATP levels in redried seeds, while in non-dried seeds it was increased resulting in significantly higher ATP values for latter. By 3 months ATP levels of stratified and redried to 25% moisture seeds were similar to control S3 but S3D1 were still lower. In source 252 the ATP levels of stratified and redried seeds were not significantly different from the controls S0, S1 and S3 at all storage periods (Fig. III.18). However, differences at 3 months were very similar to those seen in source 061.

In source 061, gametophyte ADP levels of stratified and redried to 35% moisture (S1D1, S3D1) were lower than controls S1 and S3 respectively at one and three months of storage (Fig. III.19). Gametophytes of stratified and redried seeds (S1D1, S1D2) from source 252 after one month of storage had a significantly higher ADP level than the control S1 (Fig. III.20). At zero and three months storage periods, the trend was similar but not statistically significant.

In zone 061, gametophytes of stratified and redried to 25% moisture seeds had AMP levels significantly lower than the controls S0 and S1. However, after three months of storage the AMP levels of stratified and redried seeds (S3D1, S3D2) were markedly higher than the control S3 (Fig. III.21). Gametophytes of stratified and redried to 25% moisture seeds from source 252 exhibited a larger AMP level than the control NS. After three months of storage the gametophytes

stratified and redried to 35% moisture seeds had AMP levels lower than the S3 control (Fig. III.22).

Total adenylate pool of gametophytes was significantly increased when stratified seeds were redried to 25% moisture (S0D2). Both 061 and 252 sources had similar trends (Figs. III.23 and III.24). In addition, moisture reduction to 35% was efficient in raising gametophyte TAP levels in seeds of zone 06 (S0D1). However after one month of storage the TAP levels in stratified and redried coastal seeds (S1D1, S1D2) were lower than the control S1 due to both an increase in control levels and a decrease in redried levels (Fig. III.23). In source 252 gametophytes of stratified and redried to 35% seeds (S3D1) had a TAP level lower than the control S3 after three months of storage (Fig. III.24). In contrast, gametophyte TAP level of stratified and redried to 25% seeds (S3D2) remained significantly higher than the control (S3).

Moisture reduction to 35% significantly increased gametophytes EC of stratified seeds from zone 061 (Fig. III.25). However, these seeds (S3D1) had EC lower than the control S3 after three months of storage. In seeds of interior zones, moisture reduction (S0D1, S0D2) significantly decreased gametophyte EC (Fig. III.26). After one month of storage, gametophytes of stratified and redried to 35% moisture seeds (S1D1) had an EC value lower than the control S1.

B. Nucleic acids and nucleotides

1. Effects of stratification

Stratification did not significantly affect either RNA or nucleotide levels of gametophytes from source 061 (Figs. III.27 and III.31). However, DNA levels were markedly increased during stratification (Fig. III.29). In gametophytes of zone 252, stratification increased RNA levels while the nucleotide level was reduced (Fig. III.28 and III.32). There were no differences in DNA levels between stratified seeds (S0) and nonstratified control (NS) (Fig. III.30).

2. Effects of storage following stratification

Storage significantly reduced gametophyte RNA levels of the interior source (Figure III.28). Gametophytes of source 061 had the same trend, however the differences were not statistically significant (Fig. III.27).

Three months of storage reduced gametophyte DNA levels in both 061 and 252 sources (Figs. III.29 and III.30).

Gametophyte nucleotides levels of zone 252 were lowered after three months of storage. In contrast, the gametophyte nucleotides levels of source 061 were not affected by storage conditions (Figs. III.31 and III.32).

3. Effects of moisture reduction and storage following stratification

There was no difference in gametophyte RNA levels between stratified (S0, S1, S3) and stratified and redried seeds (S0D1, S0D2,

S1D1, S1D2, S3D1, S3D2) at all storage periods (Figs. III.27 and III.28).

Gametophyte DNA levels of stratified and redried coastal seeds (S0D1, S0D2, S1D1, S1D2, S3D1, S3D2) were not significantly different than the controls (S0, S1, S3) at all storage periods (Fig. III.29). In source 252, gametophyte DNA levels of stratified and redried to 25% moisture seeds (S1D2, S3D2) were significantly different from controls S1 and S3 (Fig. III.30). After one and three months of storage the DNA level was respectively lower and higher than controls S1 and S3.

In zone 061 the gametophyte nucleotides levels of stratified, redried to 25% moisture and stored for 1 month seeds (S1D2) were significantly different from control S1 (Fig. III.31). Moisture reduction to 25% significantly increased gametophyte nucleotides levels of stratified seeds from source 252 (Fig. III.32). After 3 months of storage the stratified and redried seeds (S3D1, S3D2) gametophytes had higher nucleotides levels than S3 control.

5-Day-Old Seedlings

A. Energy status

1. Effects of stratification

ATP and ADP levels were not significantly different for seedlings originating from stratified (S0) and nonstratified (NS) seeds (Figures III.33 to III.36). The two seed sources behaved similarly.

Seedlings originated from stratified seeds (S0) from zone 061 had a significantly lower AMP level than seedlings produced by

nonstratified (NS) seeds (Fig. III.37). In zone 252, the trend was similar, but not significant (Fig. III.38).

TAP levels of seedlings produced from stratified seeds (S0) were not different from seedlings of nonstratified seeds (NS). Both sources behaved similarly (Figs. III.39 and III.40).

The seedlings originating from stratified coastal seeds (S0) had a higher EC than nonstratified control (NS) (Fig. III.41). In contrast, in zone 252, seedlings produced from stratified seeds had lower EC than nonstratified control (NS) (Fig. III.42).

2. Effects of storage following stratification

In zone 061 there was no difference in adenosine phosphate levels of seedlings produced from stratified and stored seeds (S1, S3) and those of the nonstored control (S0) (Figs. III.33, III.35 and III.37). However, in source 252 ATP and AMP levels were markedly lower in seedlings originating from seeds stratified and stored for 3 months (S3) (Figs. III.34 and III.38). Also, there was a significantly lower ADP level in seedlings produced by seeds stratified and stored for one month (S1) (Fig. III.36).

There were no differences in TAP levels or EC of seedlings produced from stratified and stored seeds (S1, S3) and those of seedlings originated from S0 control (Figs. III.39 to III.42). Both sources behaved similarly.

3. Effects of moisture reduction and storage following stratification

In general, there were no significant differences in ATP levels between seedlings produced by stratified (S0, S1, S3) and stratified and redried seeds from zone 061 (S0D1, S0D2, S1D1, S1D2, S3D1, S3D2) (Fig. III.33). However, seeds stratified, redried to 35% moisture and stored for 3 months produced seedlings with ATP level higher than the S3 control. In the interior source, stratified and redried seeds (S0D1, S0D2) produced seedlings with lower ATP levels than the stratified controls (S0) (Fig. III.34).

Stratified and redried to 25% moisture (S0D2) seeds of both sources produced seedlings with lower ADP levels than control S0 (Figs. III.35 and III.36). In addition, after one month of storage, stratified and redried to 35% moisture seeds from zone 061 yielded seedlings with lower ADP levels than the S1 control.

In source 061, the seedlings produced by stratified and redried seeds (S0D1, S0D2) had a significantly higher AMP levels than the stratified control (S0) (Fig. III.37). After one month of storage, the seedlings produced by seeds redried to 25% moisture still had higher AMP levels than the control S1. Seeds stratified, redried to 35% moisture and stored for 3 months (S3D1) produced seedlings with AMP levels higher than the nondried control (S3). In contrast, stratified and redried interior seeds (S0D1, S0D2) yielded seedlings with AMP levels lower than nondried control (S0) (Fig. III.38). However, seeds stratified, redried to 35% moisture and stored for 3 months produced seedlings with AMP levels higher than S3 control.

In source 061, the seedlings originating from stratified, redried to 35% moisture and stored for 1 month seeds had the TAP levels significantly lower than the S1 control (Fig. III.39). In contrast, after 3 months of storage these seeds yielded seedlings with TAP levels markedly higher than the control S3.

Seedlings originating from stratified and redried to 25% moisture interior seeds (S0D2) had TAP levels significantly lower than the nondried control (S0) (Fig. III.40). However, after 3 months of storage these seeds produced seedlings with TAP levels higher than the control (S3).

Redrying coastal stratified seeds to 35% moisture decreased seedling EC (Fig. III.41). Also, stratified redried to 25% moisture and stored for 3 months seeds (S3D2) yielded seedlings with EC value lower than the stratified control (S3). In contrast, redrying interior seeds to 25% moisture significantly increased seedlings EC (Fig. III.42).

B. Nucleic acids and nucleotides

1. Effects of stratification

Seedling RNA level was decreased by seed stratification in both 061 and 252 sources (Figs. III.43 and III.44). Seed stratification also reduced seedling DNA levels in source 061 (Fig. 45). Stratified coastal (S0) seeds produced seedlings with nucleotide levels higher than nonstratified control (NS) (Fig. III.47). In contrast seed stratification decreased seedling nucleotide levels in source 252 (Fig. III.48).

2. Effects of storage following stratification.

Seedling RNA levels did not differ for seedlings grown from stratified (S0) and stratified and stored seeds (S1, S3) in either source (Figs. III.43 and III.44). Also, storage of stratified seeds did not affect seedling DNA and nucleotide levels in source 061 (Figs. III.45 and III.47). However, storage of stratified interior seeds (S1, S3) increased seedling DNA and nucleotide levels (Figs. III.46 and III.48).

3. Effects of moisture reduction and storage following stratification

Seedlings originating from stratified and redried to 35% moisture seeds (S0D1) of zone 061 had significantly higher RNA levels than the stratified control (S0) (Fig. III.43). In source 252, redrying stratified seeds did not significantly change seedling RNA levels at all storage periods (Fig. III.44).

Stratified and redried to 25% moisture seeds from zone 061 (S0D2, S1D2) yielded seedlings with DNA levels lower than the stratified nonstored (S0) and the stratified and stored for 1 month (S1) controls (Fig. III.45). In source 252, redrying stratified seeds to 35% moisture (S0D1) increased seedlings DNA levels (Fig. III.46). Seedlings originating from stratified seeds redried to 25% moisture and stored for 3 months had DNA levels lower than the control S3.

In source 061, the seedlings originating from stratified and redried to 25% moisture seeds (S0D2, S3D2) had nucleotide levels consistently lower than the controls S0 and S3 at nonstorage and three

month of storage periods respectively (Fig. III.47). In source 252 stratified and redried to 35% moisture seeds (S0D1) yielded seedlings with nucleotide levels significantly higher than the control S0 (Fig. III.48). However, after 3 months of storage, stratified and redried to 25% moisture seeds produced seedlings with nucleotide levels lower than the nondried control (S3).

Discussion and Conclusions

Stratification improved the energy status of seeds from both 061 and 252 sources by increasing ATP in the embryo to 13-fold and in the gametophyte to 6-fold of the original quantity. Energy charge rose to values above .8 which are characteristic of actively metabolizing tis sues (Pradet and Raymond 1983). Similarly Ching and Ching (1972, 1973), Szczotka and Tomazenska (1981) and Murphy and Noland (1982) reported marked increase in energy metabolism during stratification of Ponderosa pine, Douglas-fir, Norway maple (Acer platanoides L.) and sugar pine (Pinus lambertiana Dougl.) seeds. The increase in ATP in embryos and gametophytes was accompanied by an increase in the total pool of adenosine phosphates, which suggests an active de novo synthesis be-sides the regeneration pathways (e.g. oxidative phosphorylation, sub-strate level phosphorylation) (Ching 1982). Changes in the other two species of adenosine phosphates differed in direction between seed sources and in magnitude between embryos and gametophytes. This diversity of trends is perhaps due to the genetic variation between sources and to the divergent metabolic activities of embryos and gametophytes.

Stratification markedly increased RNA levels in embryos and gametophytes of both seed sources. Similar findings were reported by a number of investigators in seeds of Douglas-fir (Ching 1966), hazel (Corylus avellana L.) (Wood and Bradbeer 1967) and Norway maple (Davies and Pinfield 1979, Slater and Bryant 1982). In this study embryo DNA and nucleotide levels were unchanged by stratification. These results contrast with the findings of Jarvis et al. (1968) and Ching (1966). Perhaps the differences can be attributed to the variation in the species and seed sources used in respective studies. Possibly the difference between Ching's results and these may be explained by the existence of ecotypic variations in physiological behavior (Allen 1960). The results of this study indicate a small DNA increase and nucleotide decrease in the gametophytes of both seed sources. These findings are in general agreement with Ching's (1966) report on Douglas-fir seeds germinational pattern.

High phosphorylative efficiency and high RNA levels of stratified seeds correlate with the fact of fast seed germination reported in Chapter II. Therefore, with support in the literature (Ching 1966, Ching and Ching 1972, 1973; Jarvis et al. 1968; Slater and Bryant 1982) we assumed that the biochemical changes observed here were part of the stratification effects which alters dormant seed metabolism in a particular direction leading to the relative stimulation of a process, as yet unknown, essential for the breaking of dormancy. Thus, further investigation into the stability of these stratification biochemical benefits in relation to subsequent redrying and storage were conducted in order to discern the effects of these processes.

Moisture reduction of stratified seeds definitely increased embryo RNA and DNA levels. Koehler (1967) showed an increased rate of respiration and increase in levels of proteins and RNA in tomato seeds as a result of osmotic pretreatment. More recently, Sen and Osborne (1974) and Dell'Aquilla et al. (1978) reported that the hydration-dehydration treatment enhanced the ability of cereal embryos to synthesize protein, RNA and DNA, during the early hours of germination. There are similarities between those findings and the biochemical responses observed in this study, assuming that dormancy had been broken before the redrying treatment. In this study, redrying seems to shift both the rate of nucleic acid synthesis and, the rate of germination in parallel fashion. In addition, redrying produces more vigorous seedlings (Chapter II). Therefore, the data suggest a possible causal relationship between these biochemical and physiological processes. However, more detailed studies should be conducted in exploring the mechanism(s) of partial dehydration of hydrated tissues resulting in enhanced synthesis of protein RNA and DNA at the early germination stages of seeds.

Seed moisture reduction didn't significantly affect nucleic acids and nucleotide levels of gametophytes. The varying responses between embryos and gametophytes can possibly be attributed to the fact that protein synthesis in the latter is mostly unrelated to germination per se, but instead to the mobilization of stored reserves which is a post germination phenomenon (Bewley 1982).

Embryo adenosine triphosphate levels and the energy charge of stratified seeds were stable during redrying. Possibly, ATP, RNA's and nucleotides had a parallel increase during seed moisture

reduction. However, due to the rapid turnover of cell energy and a major demand of ATP for nucleic acid and nucleotide synthesis (Ching 1982) a steady energy state and ATP pool were observed. Patterns of change in levels of the other two adenosine species were different in magnitude and direction for the two seed sources. The adenosine phosphate levels in the storage tissue varied in a nonconsistent fashion within and between seed sources. The general pattern indicated an increase in ATP and energy charge in coastal seeds. In the interior source, gametophytes ATP levels weren't affected by redrying, however, there was a reduction in energy charge. This fact probably indicates that the utilization of ATP exceeded biosynthesis. Such differences between sources could be the variation in the metabolic state in which seeds were arrested during development and drying, or upon conditions under which seeds were extracted and stored, or there might simply be genetical differences. Nevertheless, TAP levels were preserved during redrying in embryos and gametophytes of both sources.

Storage of stratified seeds of both seed sources for 1 or 3 months markedly lowered RNA levels of embryos and gametophytes indicating an enhanced RNase activity during the low temperature storage. No quantitative difference was observed in embryo DNA and nucleotides levels, although they were slightly reduced in the storage tissues. Studies reported in Chapter II indicated that vigor and viability of stratified seeds were also reduced throughout storage. Several papers report that aging reduced seed vigor in cereal and dicotyledone seeds as measured by the ability for RNA, DNA and protein synthesis (Van Onckelen et al. 1974, Anderson 1977, Ching 1973a, b). However, no quantitative changes in total RNA and DNA acid levels was observed

during loss of viability (Ching 1972, Osborne 1982), but qualitative changes were reported. Therefore, enzymatic activity is an important aspect in the seed or seedling vigor in studies of this type. In both seed sources, storage of stratified seeds at low temperature reduced ATP level, TAP level, energy charge, and quantity of RNA in parallel fashion. Such parallel reduction in energy status, RNA synthetic ability and seed vigor has been reported for barley, soybean and crimson clover (Van Onckelen et al. 1974, Anderson 1977 and Ching 1973a, b). Perhaps the low vigor of these seeds after storage may be explained by an impairment in the ability of utilization rather than synthesis of ATP. Storage also reduced ATP and TAP levels of gametophytes, however the energy charge was preserved. The patterns of ADP and AMP in gametophytes were not consistent enough to discuss.

Generally, the loss of benefits of stratification and subsequent deterioration throughout storage were similar for stratified and stratified and redried seeds.

None of the biochemical criteria studied in 5-day-old seedlings showed close proportionality with either physiological responses previously reported or the biochemical changes obtained in the seed stage. In addition, the seedling data have an inconsistent pattern that does not allow the suggestion that the treatments applied in the seed stage had uniform effect on the general metabolism of 5-day-old seedling of different seed sources. Possibly seedlings morphological and biochemical development were not in pace among different treatments thus resulting in varied quantity of biochemicals. Perhaps older seedlings will express the stratification, redrying and storage effect in a more efficient fashion.

We conclude from these and the results in Chapter II that maybe there is a metabolic improvement induced by seed moisture reduction in addition to the preservation of activated metabolic processes that occurred during stratification. However, these benefits are not stable after storage for 1 or 3 months, therefore it would be advantageous to redry stratified seeds before sowing to produce vigorous seedings or to allow the expression of best benefits of stratification. Further studies are needed to investigate the use of this technique on a production basis paying special attention to provenances, maturity and processing procedures.

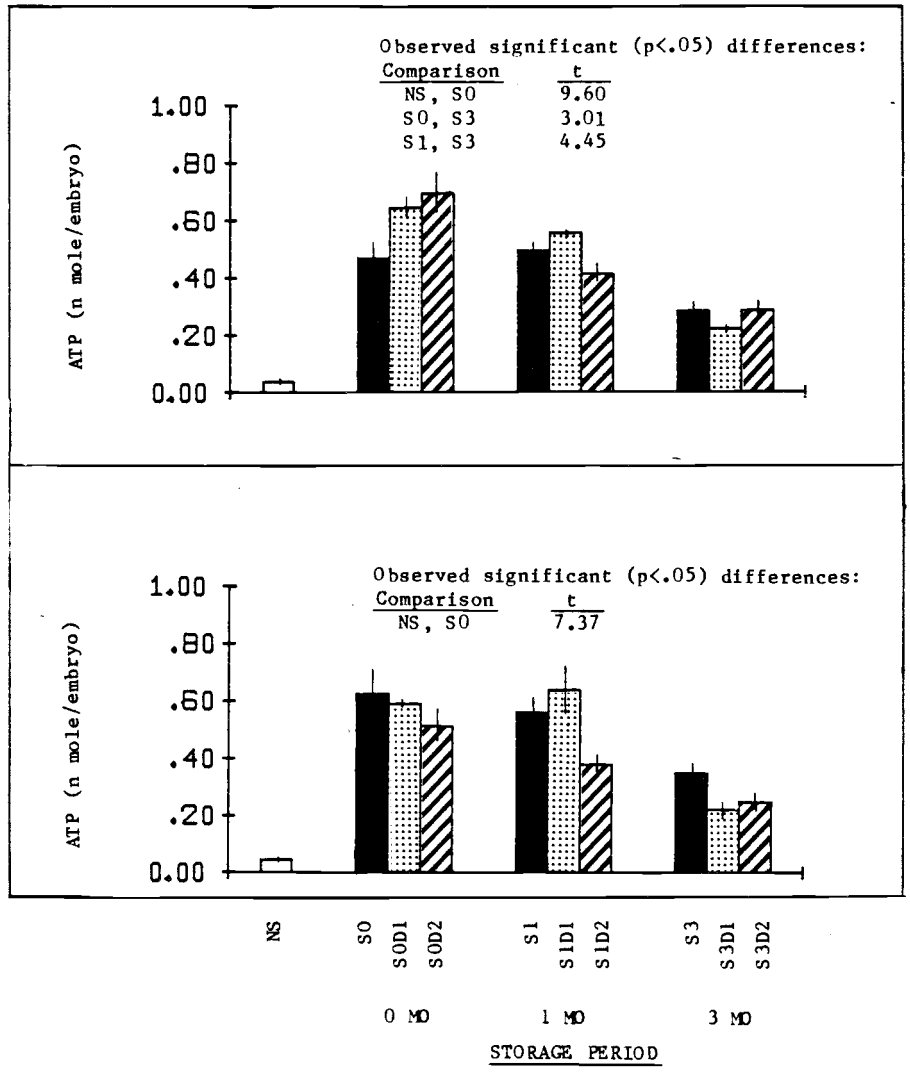
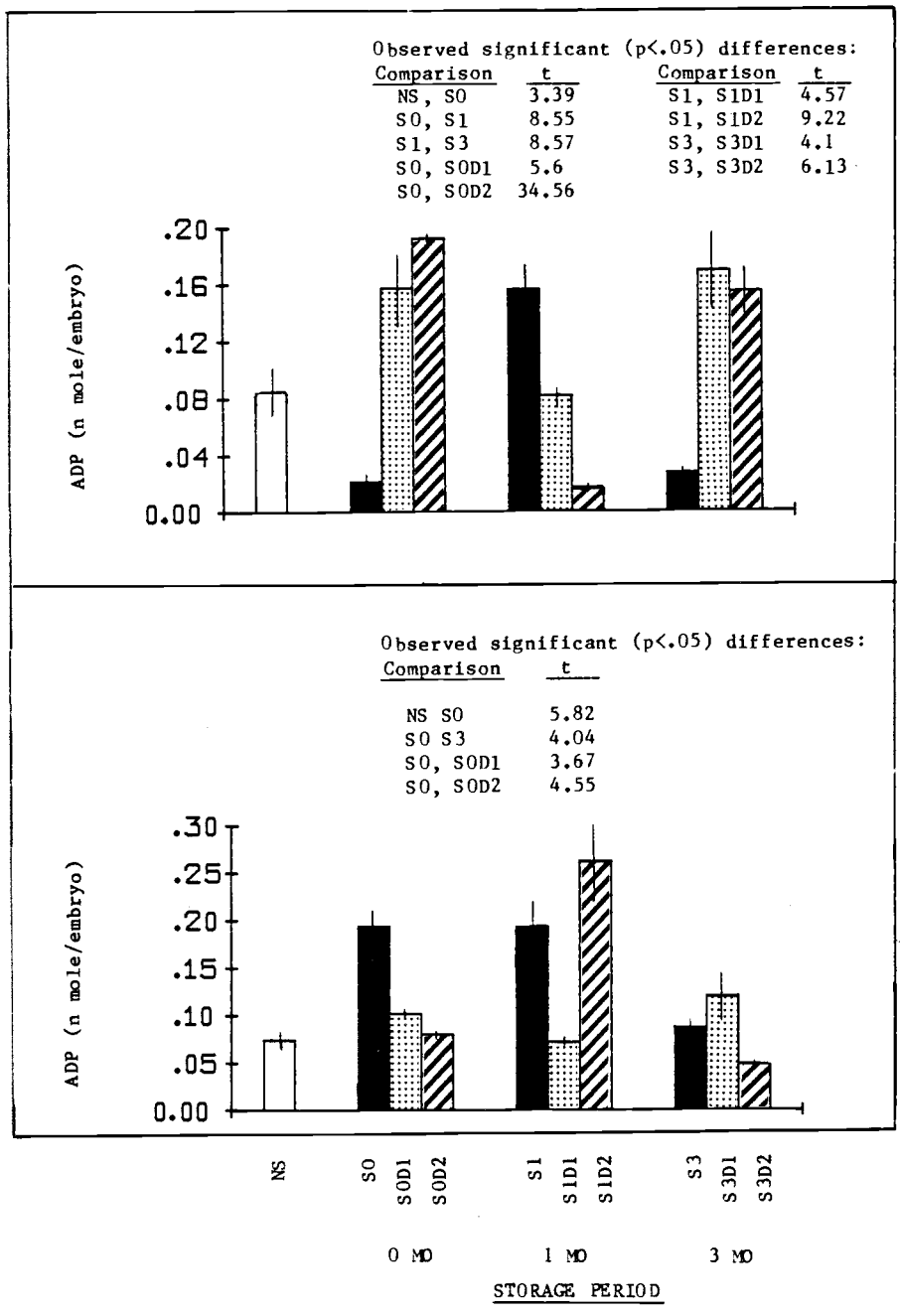
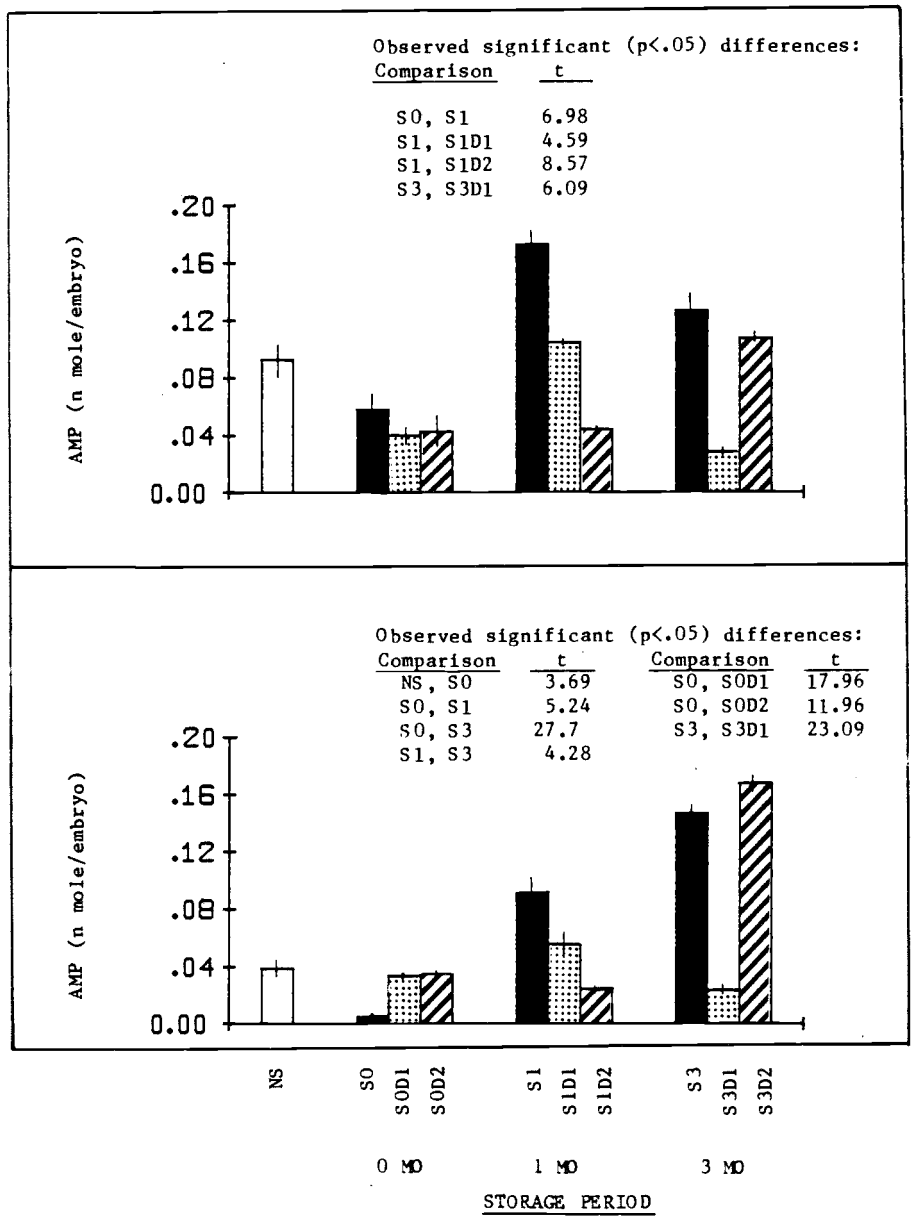


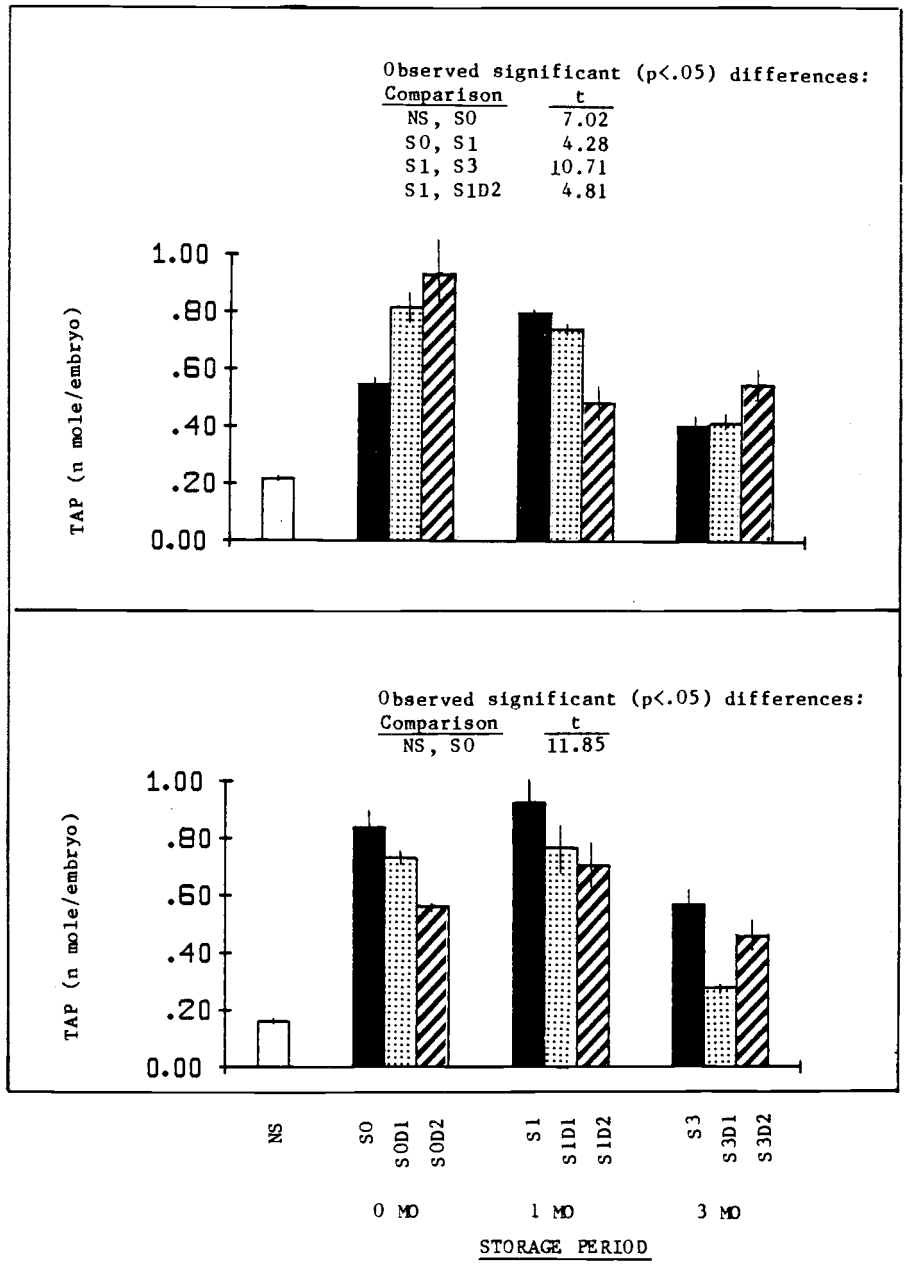
Figure III.1 and III.2. Effect of storage period and moisture content on embryo ATP levels of stratified Pseudotsuga menziesii seeds from zones 061 (Figure III.1) and 252 (Figure III.2) ■ - 45% moisture content, ⁞⁞⁞ - 35% moisture content, ▨ - 25% moisture content. Bars represent mean ATP levels of 3 replications of 20 embryos. Error bars represent standard errors.



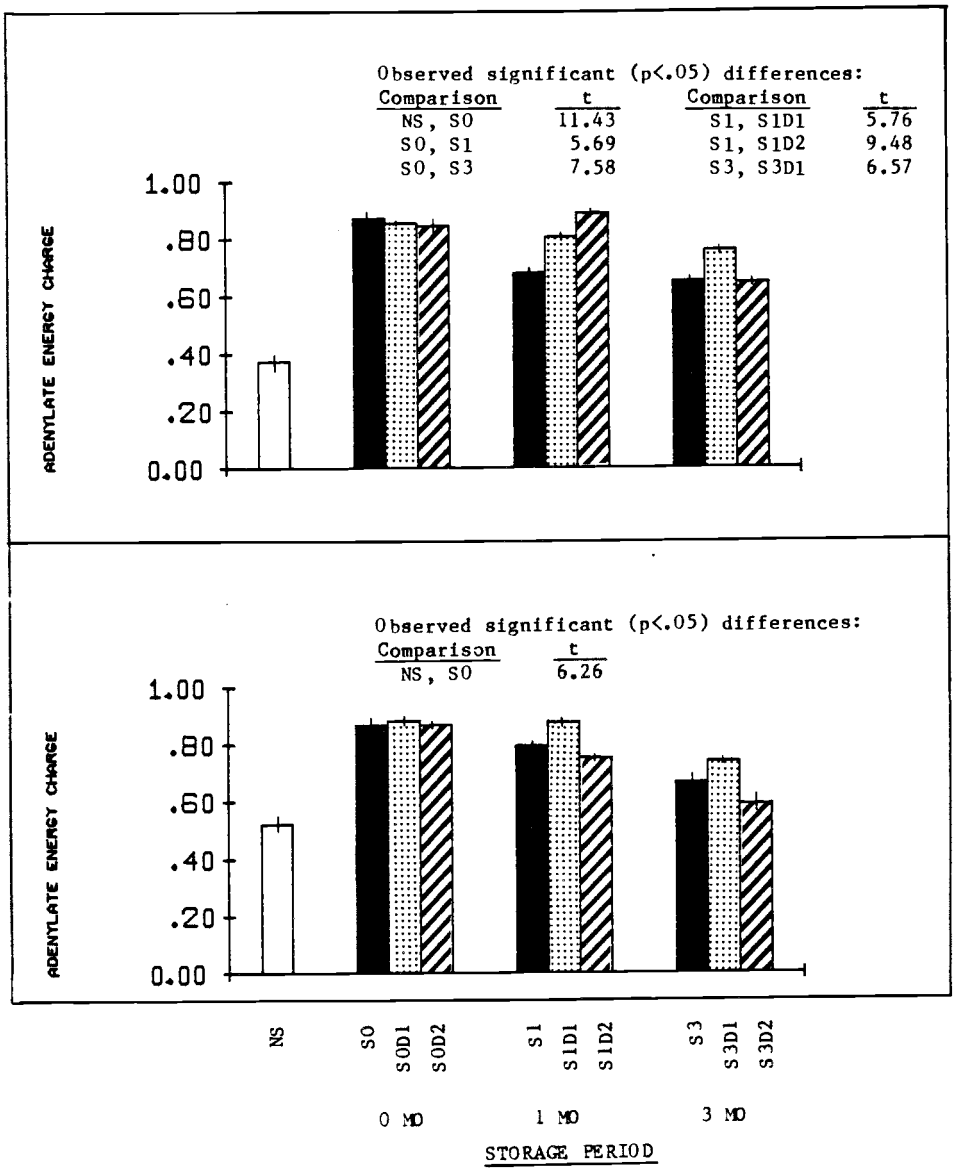
Figures III.3 and III.4. Effect of storage period and moisture content on embryo ADP levels of stratified Pseudotsuga menziesii seeds from zones 061 (Fig. III.3) and 252 (Fig. III.4). ■ - 45% moisture content, ⁞⁞ -35% moisture content, ▨ - 25% moisture content. Bars represent mean ADP levels of 3 replications of 20 embryos. Error bars represent standard errors.



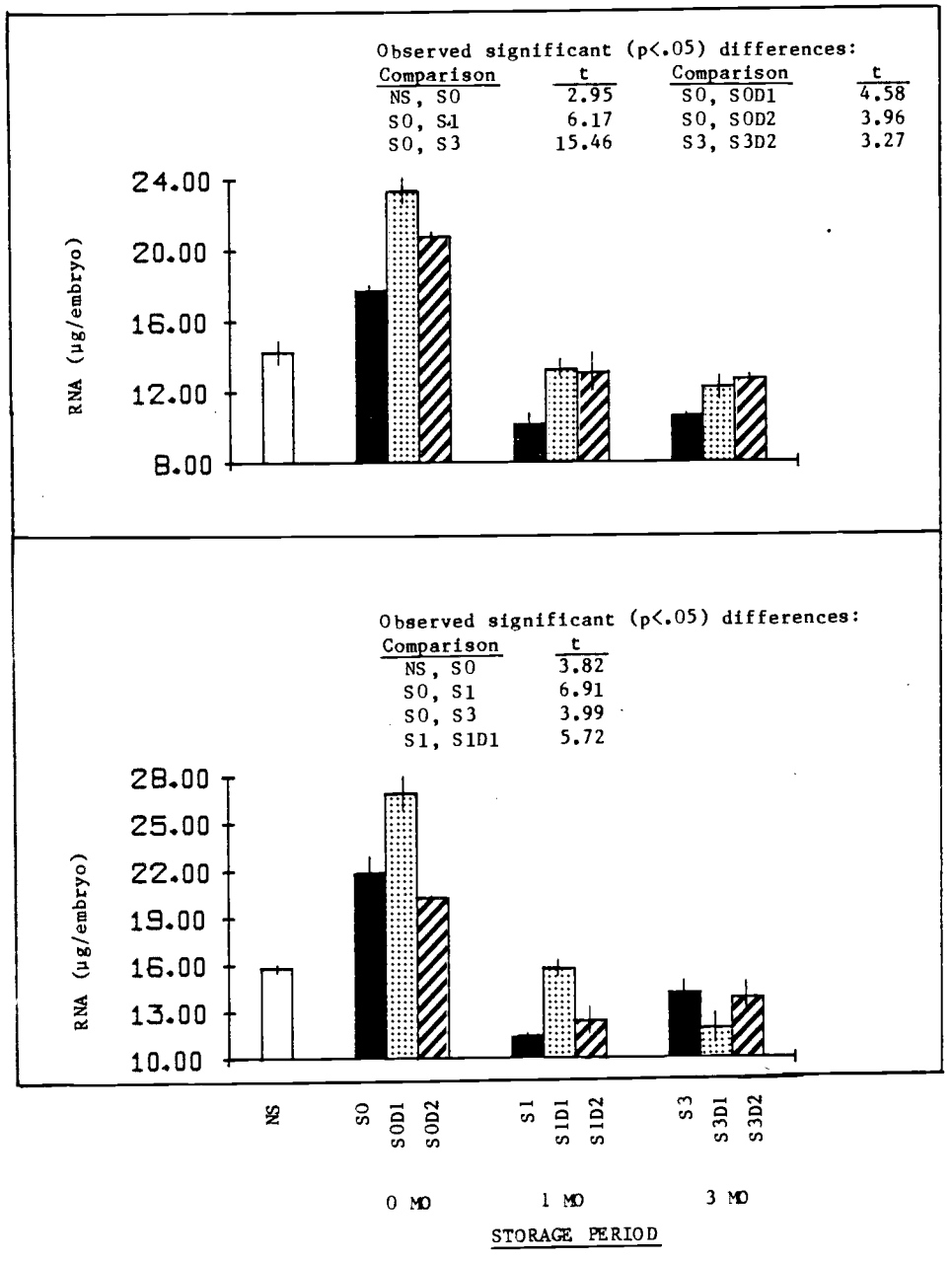
Figures III.5 and III.6. Effect of storage period and moisture content on embryo AMP levels of stratified Pseudotsuga menziesii seeds from zones 061 (Fig. III.5) and 252 (Fig. III.6). ■ - 45% moisture content, ⁞⁞⁞ - 35% moisture content, ▨ - 25% moisture content. Bars represent mean AMP levels of 3 replications of 20 embryos. Error bars represent standard errors.



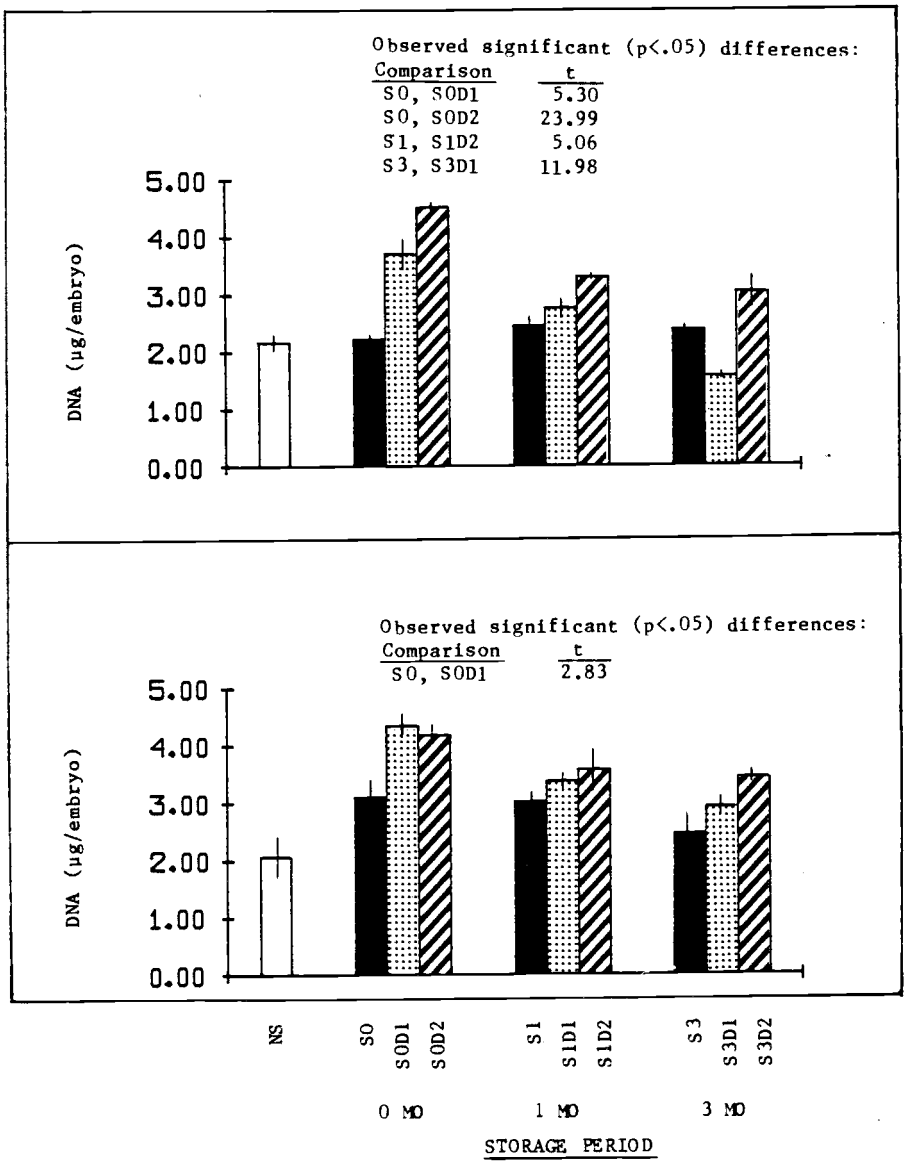
Figures III.7 and III.8. Effect of storage period and moisture content on embryo TAP levels of stratified Pseudotsuga menziesii seeds from zones 061 (Fig. III.7) and 252 (Fig. III.8). ■ - 45% moisture content, ⁞⁞ - 35% moisture content, ▨ - 25% moisture content. Bars represent mean TAP levels of 3 replications of 20 embryos. Error bars represent standard errors.



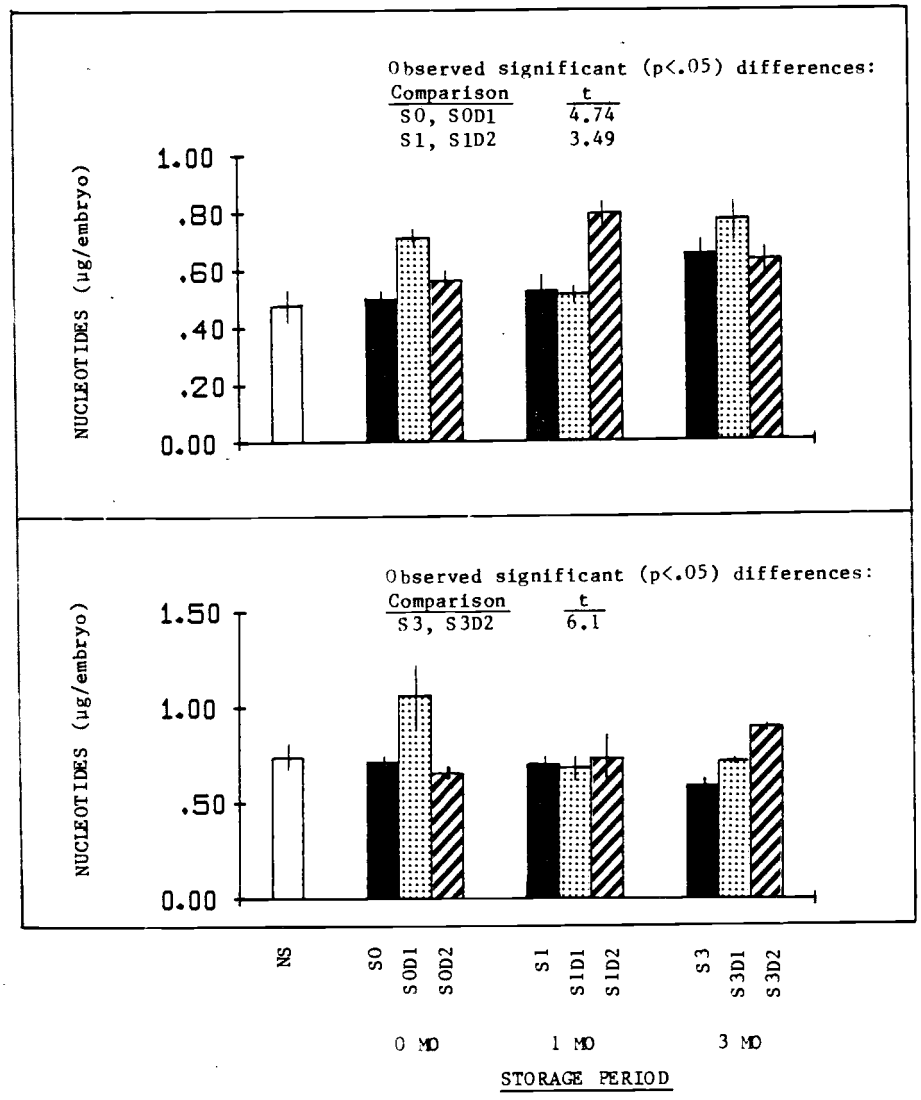
Figures III.9 and III.10. Effect of storage period and moisture content on embryo adenylate energy charge of stratified Pseudotsuga menziesii seeds from zones 061 (Fig. III.9) and 252 (Fig. III.10). ■ - 45% moisture content, ⁙ - 35% moisture content, ▨ - 25% moisture content. Bars represent mean adenylate energy charge of 3 replications of 20 embryos. Error bars represent standard errors.



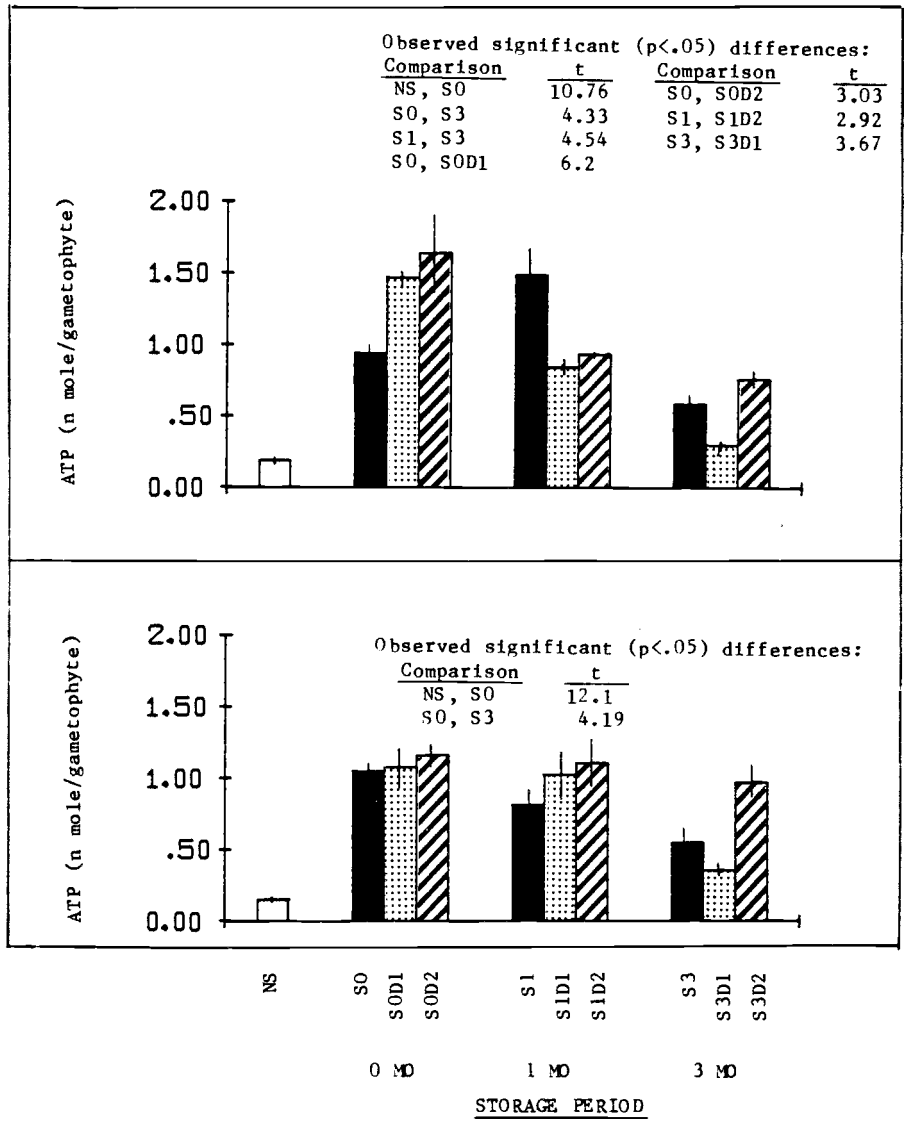
Figures III.11 and III.12. Effect of storage period and moisture content on embryo RNA levels of stratified Pseudotsuga menziesii seeds from zones 061 (Fig. III.11) and 252 (Fig. III.12). ■ - 45% moisture content, ⁙ - 35% moisture content, ▨ - 25% moisture content. Bars represent mean RNA levels of 3 replications of 20 embryos. Error bars represent standard errors.



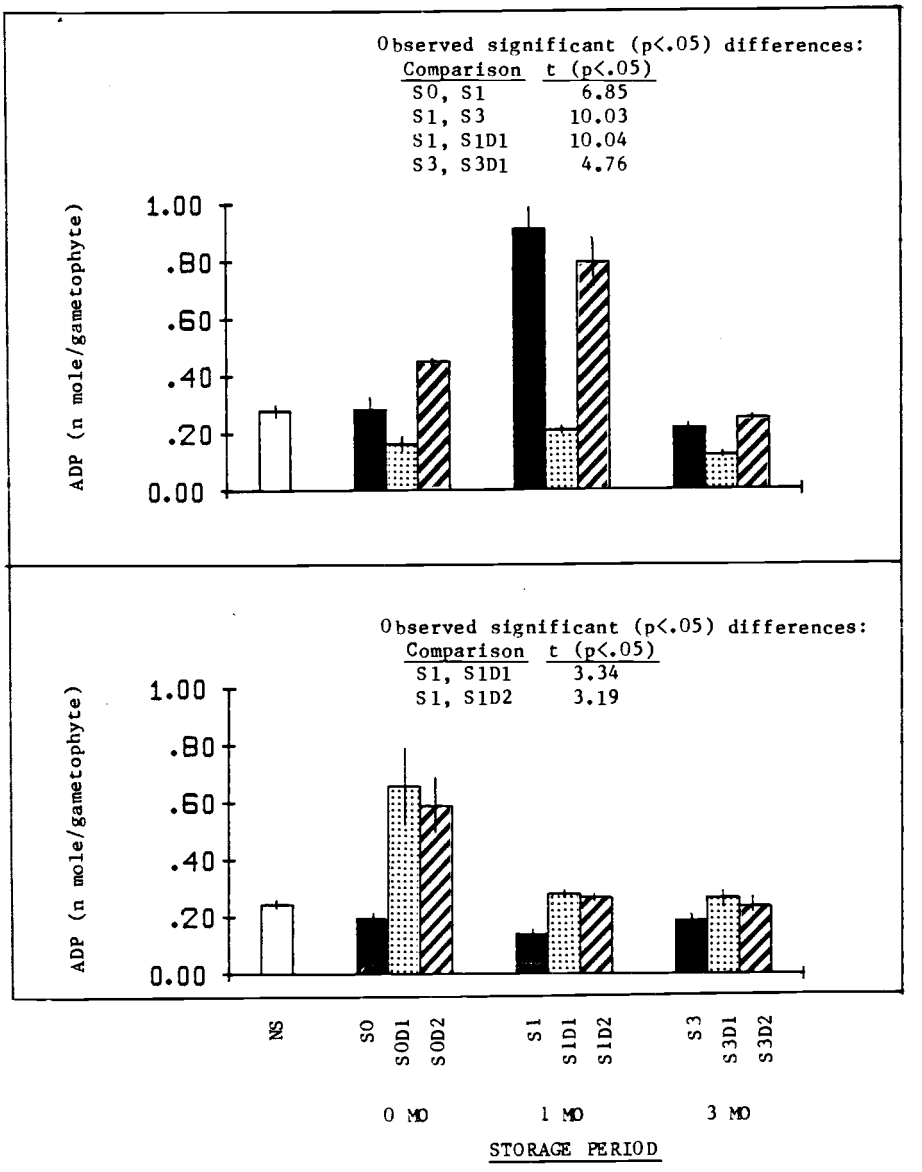
Figures III.13 and III.14. Effect of storage period and moisture content on embryo DNA levels of stratified Pseudotsuga menziesii seeds from zones 061 (Fig. III.13) and 252 (Fig. III.14). ■ - 45% moisture content, ⁙ - 35% moisture content, ▨ - 25% moisture content. Bars represent mean DNA levels of 3 replications of 20 embryos. Error bars represent standard errors.



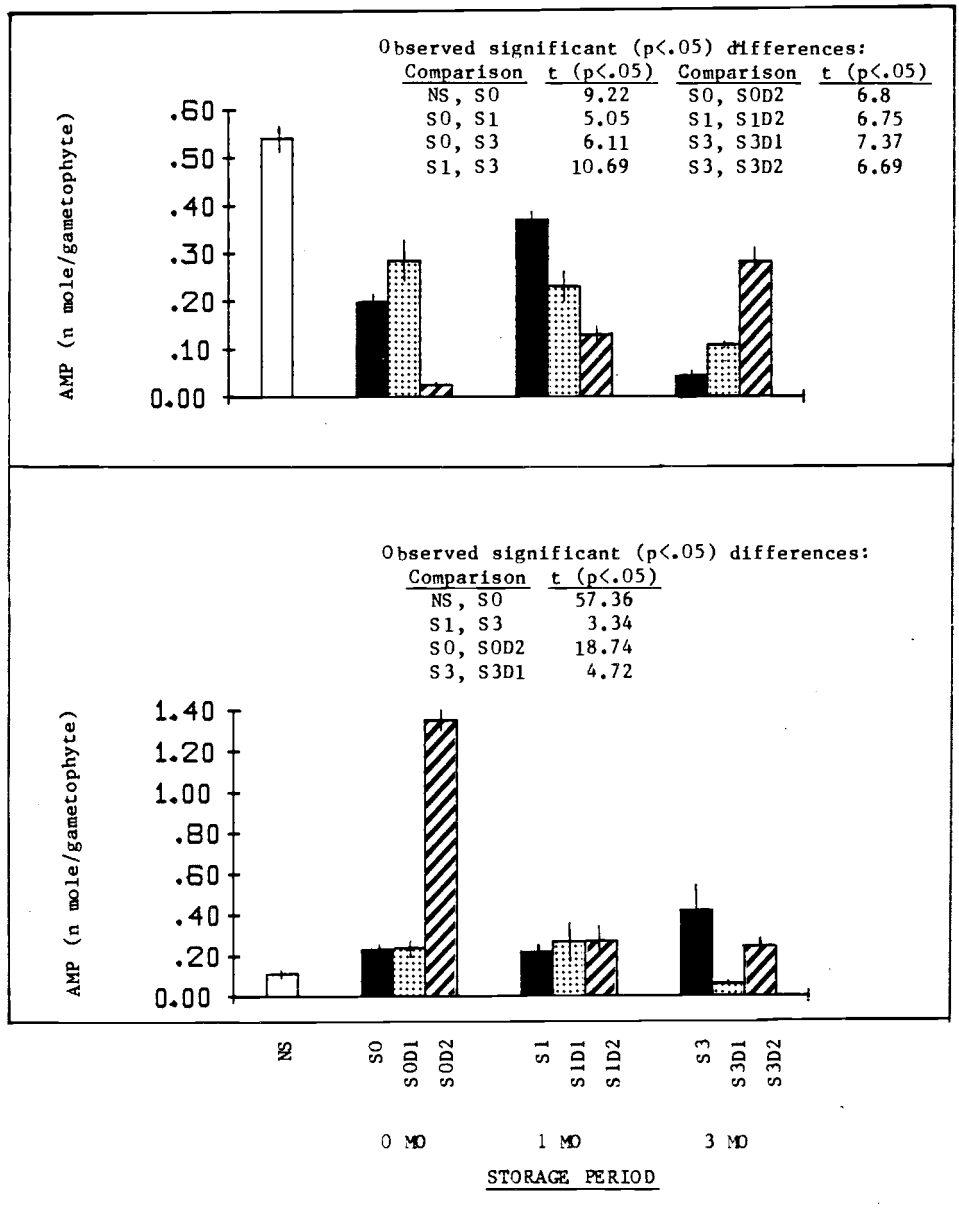
Figures III.15 and III.16. Effect of storage period and moisture content on embryo nucleotides levels of stratified Pseudotsuga menziesii seeds from zones 061 (Fig. III.15) and 252 (Fig. III.16). ■ - 45% moisture content, ⁙ -35% moisture content, ▨ - 25% moisture content. Bars represent mean nucleotides levels of 3 replications of 20 embryos. Error bars represent standard errors.



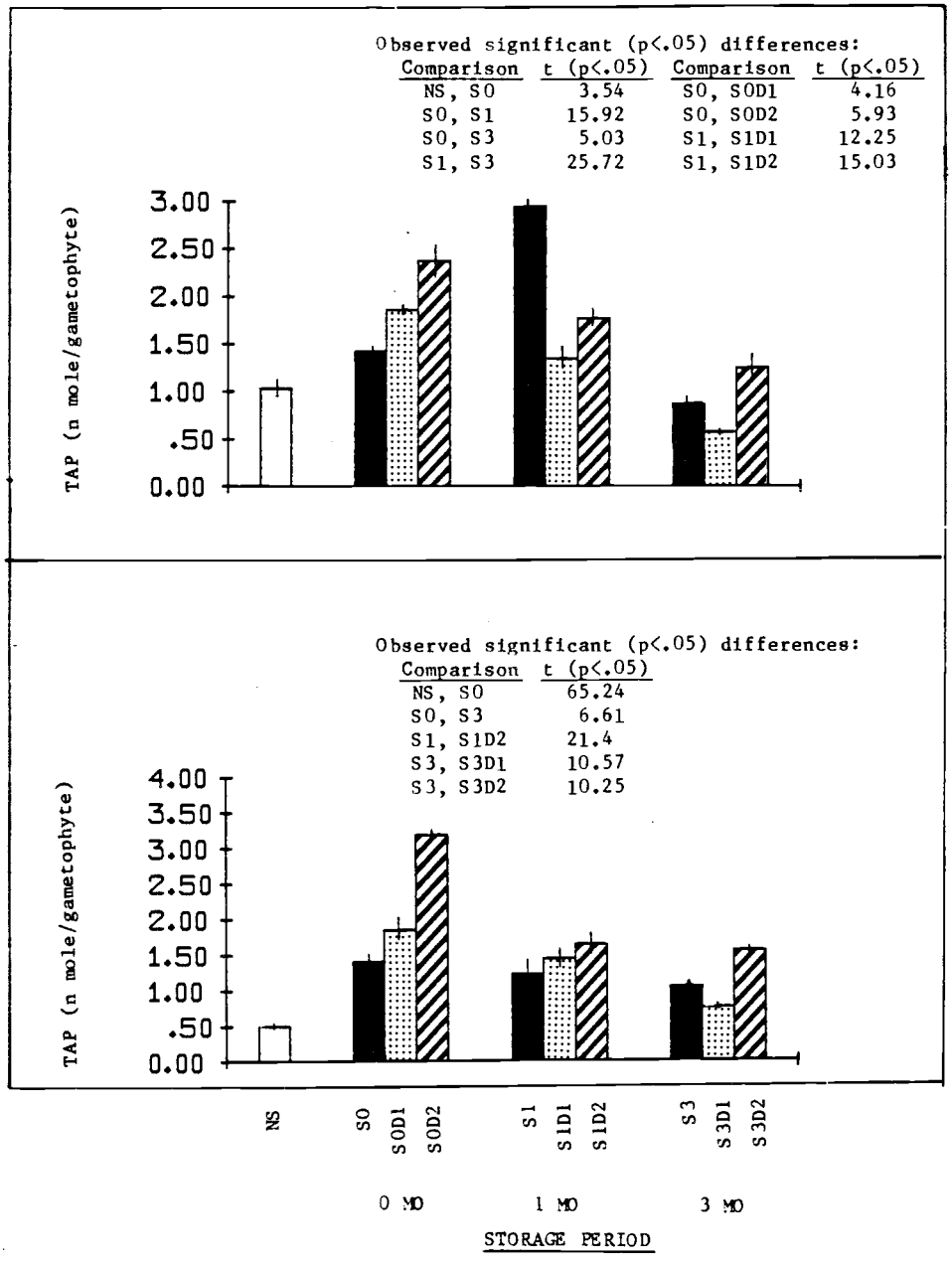
Figures III.17 and III.18. Effect of storage period and moisture content on gametophyte ATP levels of stratified Pseudotsuga menziesii seeds from zones 061 (Fig. III.17) and 252 (Fig. III.18). ■ - 45% moisture content, ⁞⁞⁞ - 35% moisture content, ▨ - 25% moisture content. Bars represent mean ATP levels of 3 replications of 20 gametophytes. Error bars represent standard errors.



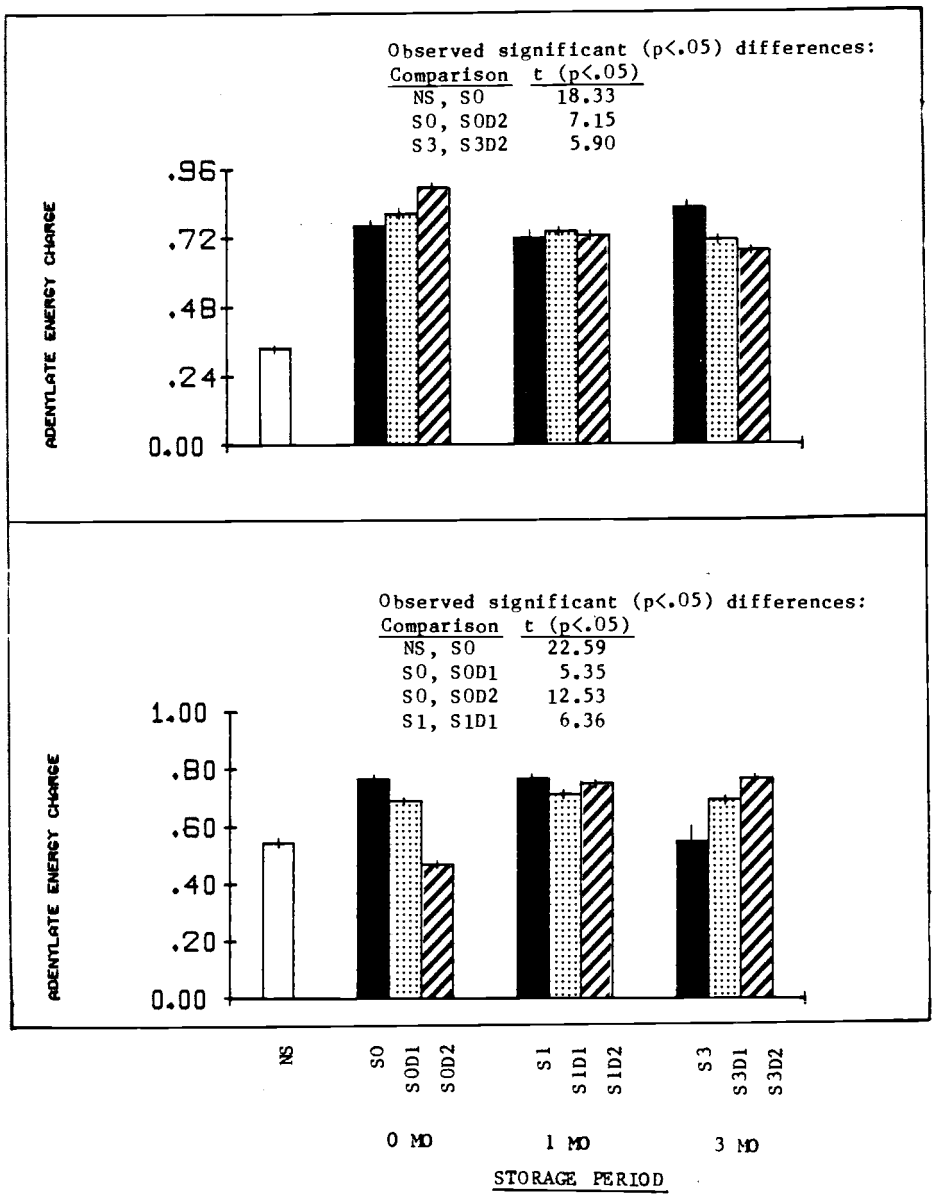
Figures III.19 and III.20. Effect of storage period and moisture content on gametophyte ADP levels of stratified Pseudotsuga menziesii seeds from zones 061 (Fig. III.19) and 252 (Fig. III.20). ■ - 45% moisture content, ⁞⁞ - 35% moisture content, ▨ - 25% moisture content. Bars represent mean ADP levels of 3 replications of 20 gametophytes. Error bars represent standard errors.



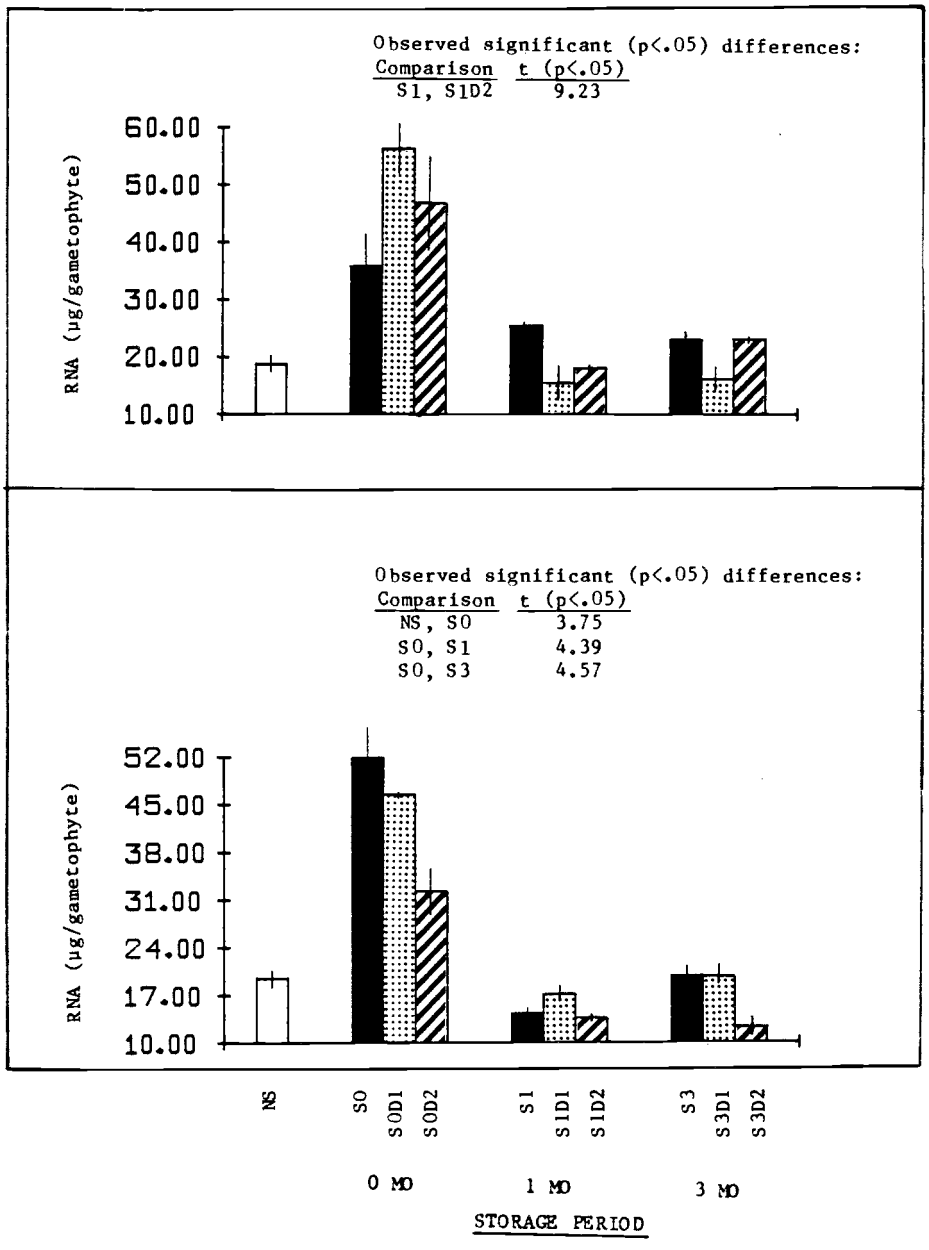
Figures III.21 and III.22. Effect of storage period and moisture content on gametophyte AMP levels of stratified Pseudotsuga menziesii seeds from zones 061 (Fig. III.21) and 252 (Fig. III.22). ■ - 45% moisture content, ⁞⁞ - 35% moisture content, ▨ - 25% moisture content. Bars represent mean AMP levels of 3 replications of 20 gametophytes. Error bars represent standard errors.



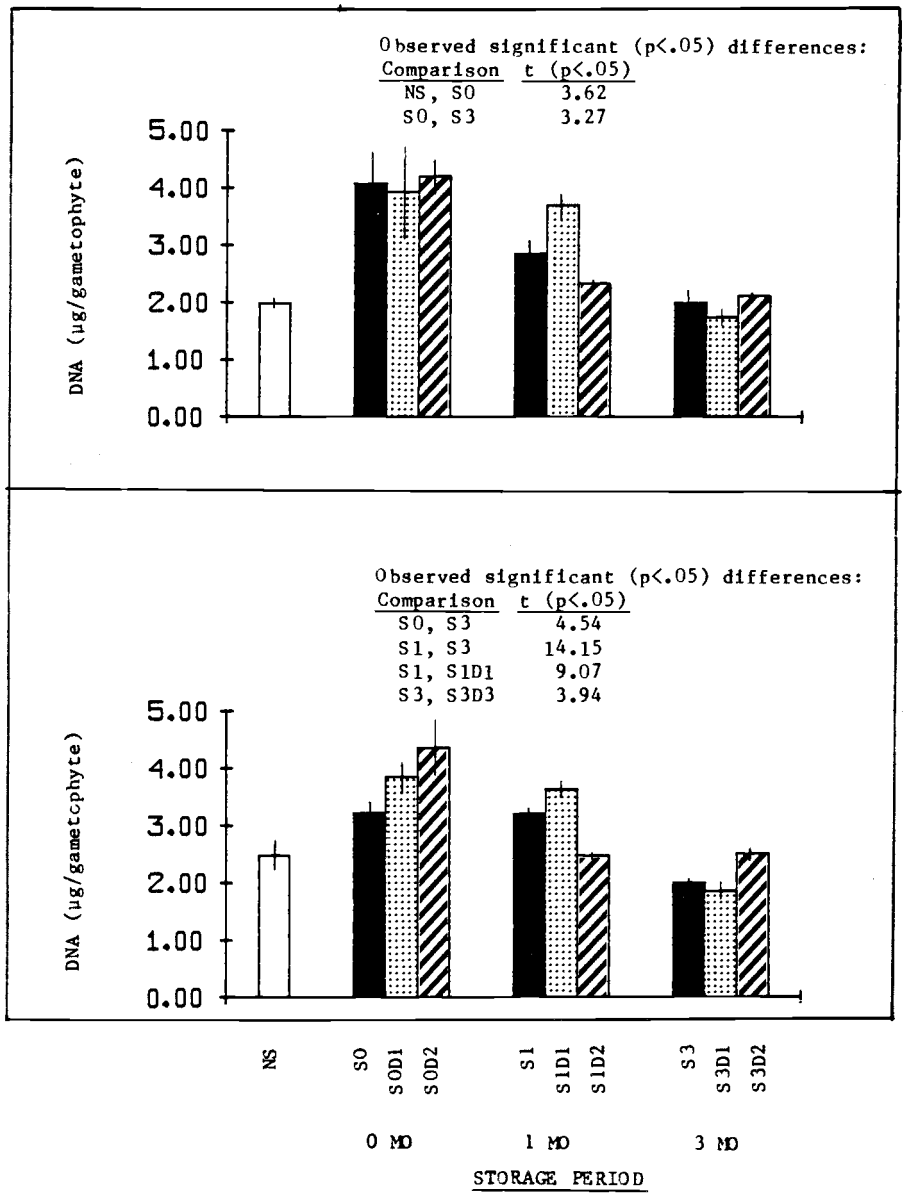
Figures III.23 and III.24. Effect of storage period and moisture content on gametophyte TAP levels of stratified Pseudotsuga menziesii seeds from zones 061 (Fig. III.23) and 252 (Fig. III.24). ■ - 45% moisture content, ⁞⁞⁞ - 35% moisture content, ▨ - 25% moisture content. Bars represent mean TAP levels of 3 replications of 20 gametophytes. Error bars represent standard errors.



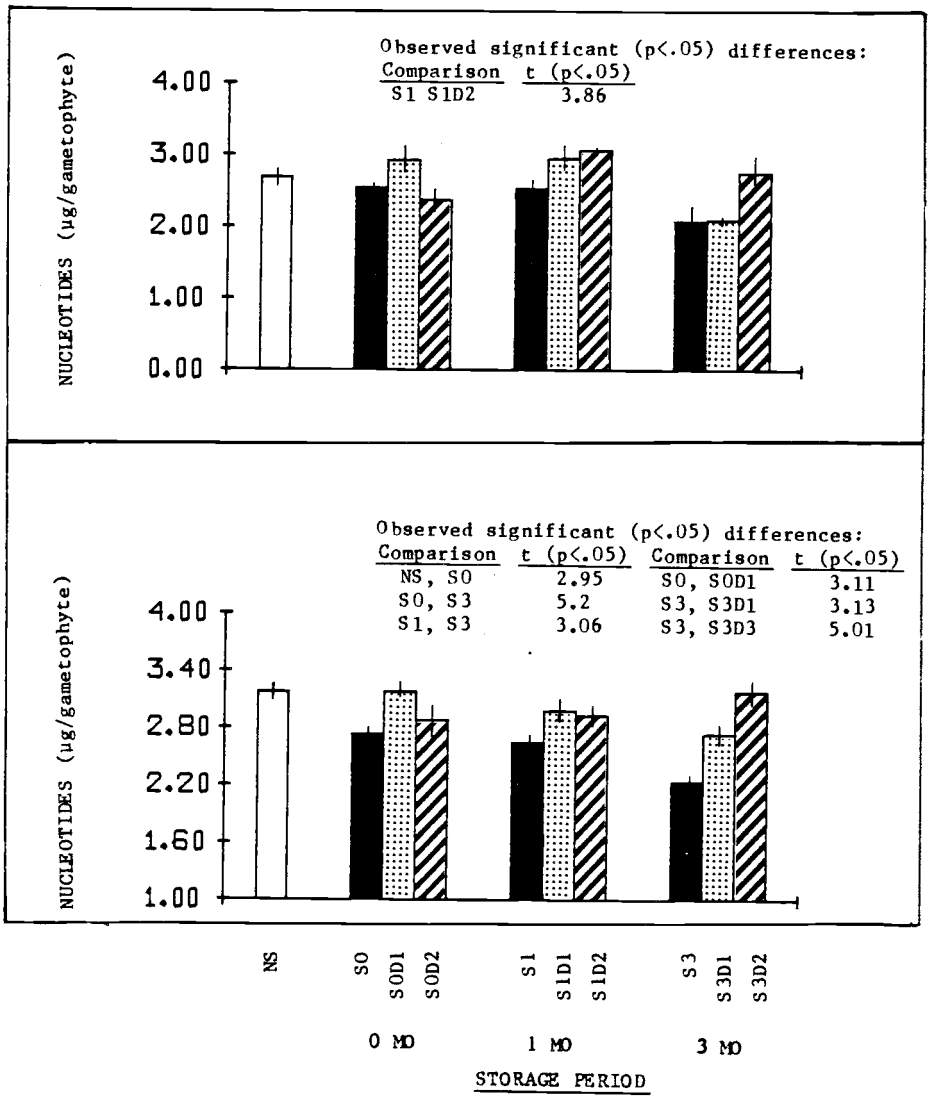
Figures III.25 and III.26. Effect of storage period and moisture content on gametophyte adenylate energy charge of stratified Pseudotsuga menziesii seeds from zones 061 (Fig. III.25) and 252 (Fig. III.26). ■ - 45% moisture content, ⁞⁞ - 35% moisture content, ▨ - 25% moisture content. Bars represent mean adenylate energy charge of 3 replications of 20 gametophytes. Error bars represent standard errors.



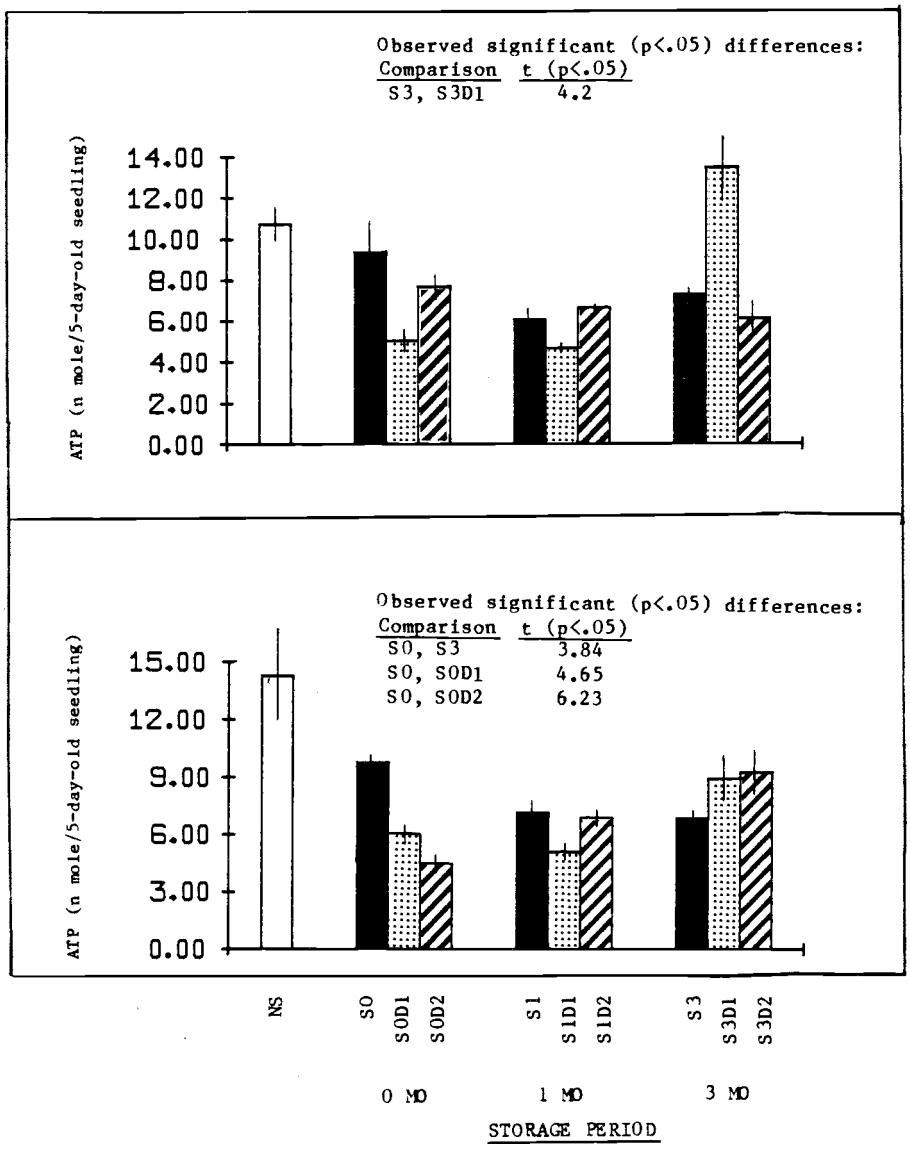
Figures III.27 and III.28. Effect of storage period and moisture content on gametophyte RNA levels of stratified Pseudotsuga menziesii seeds from zones 061 (Fig. III.27) and 252 (Fig. III.28). ■ - 45% moisture content, ⁞⁞ - 35% moisture content, ▨ - 25% moisture content. Bars represent mean RNA levels of 3 replications of 20 gametophytes. Error bars represent standard errors.



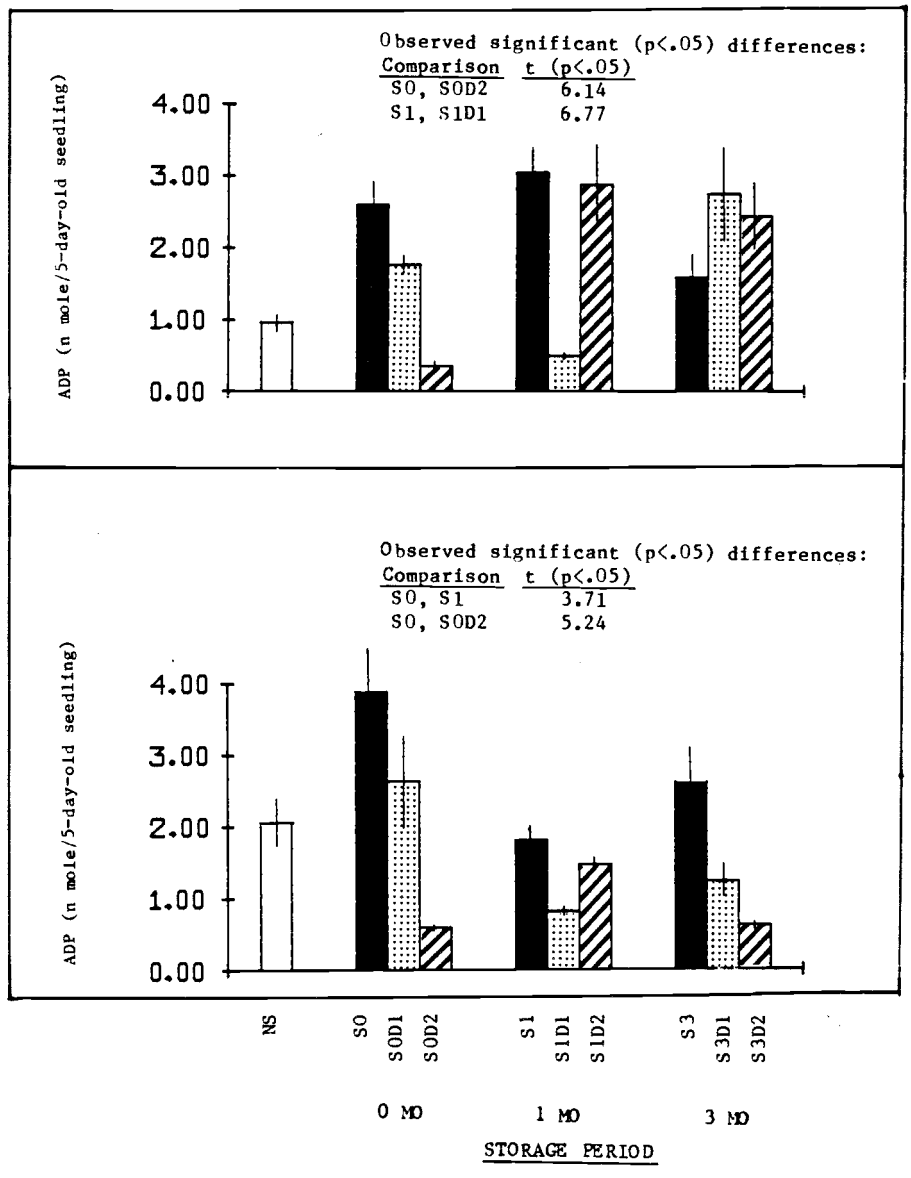
Figures III.29 and III.30. Effect of storage period and moisture content on gametophyte DNA levels of stratified Pseudotsuga menziesii seeds from zones 061 (Fig. III.29) and 252 (Fig. III.30). ■ - 45% moisture content, ⁙ - 35% moisture content, ▨ - 25% moisture content. Bars represent mean DNA levels of 3 replications of 20 gametophytes. Error bars represent standard errors.



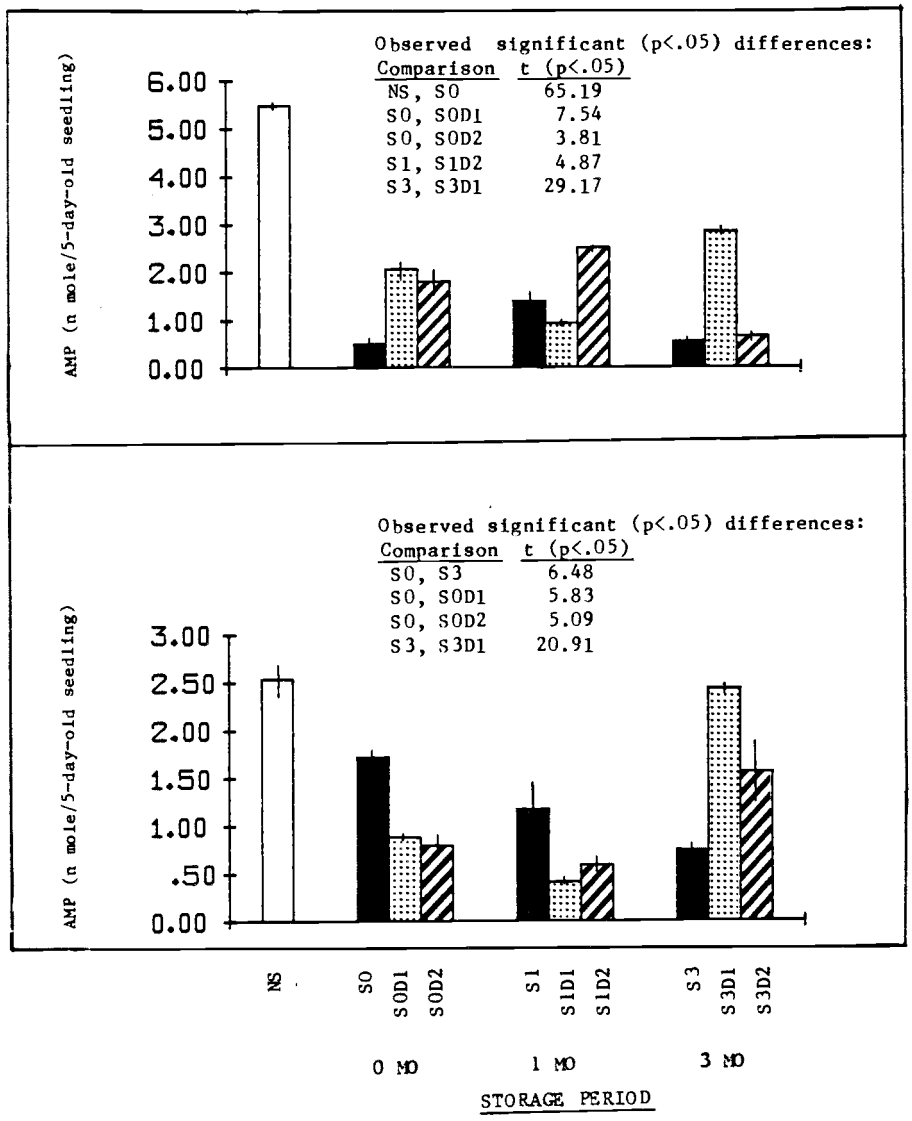
Figures III.31 and III.32. Effect of storage period and moisture content on gametophyte nucleotides levels of stratified Pseudotsuga menziesii seeds from zones 061 (Fig. III.31) and 252 (Fig. III.32). ■ - 45% moisture content, ⁙ - 35% moisture content, ▨ - 25% moisture content. Bars represent mean nucleotides levels of 3 replications of 20 gametophytes. Error bars represent standard errors.



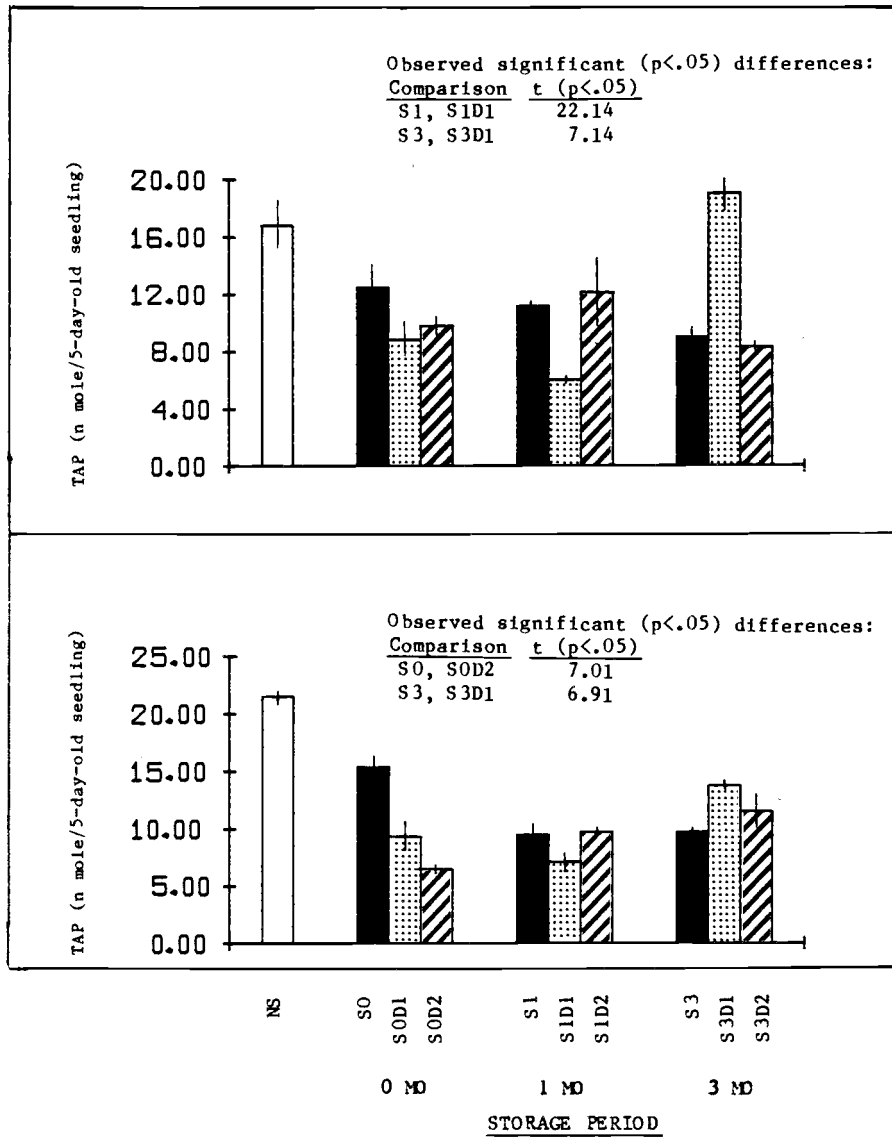
Figures III.33 and III.34. Effect of storage period and moisture content on ATP levels of 5-day-old seedlings originating from stratified Pseudotsuga menziesii seeds from zones 061 (Fig. III.33) and 252 (Fig. III.34). ■ - 45% moisture content, ⁞⁞⁞ - 35% moisture content, ▨ - 25% moisture content. Bars represent mean ATP levels of 3 replications of 4 seedlings. Error bars represent standard errors.



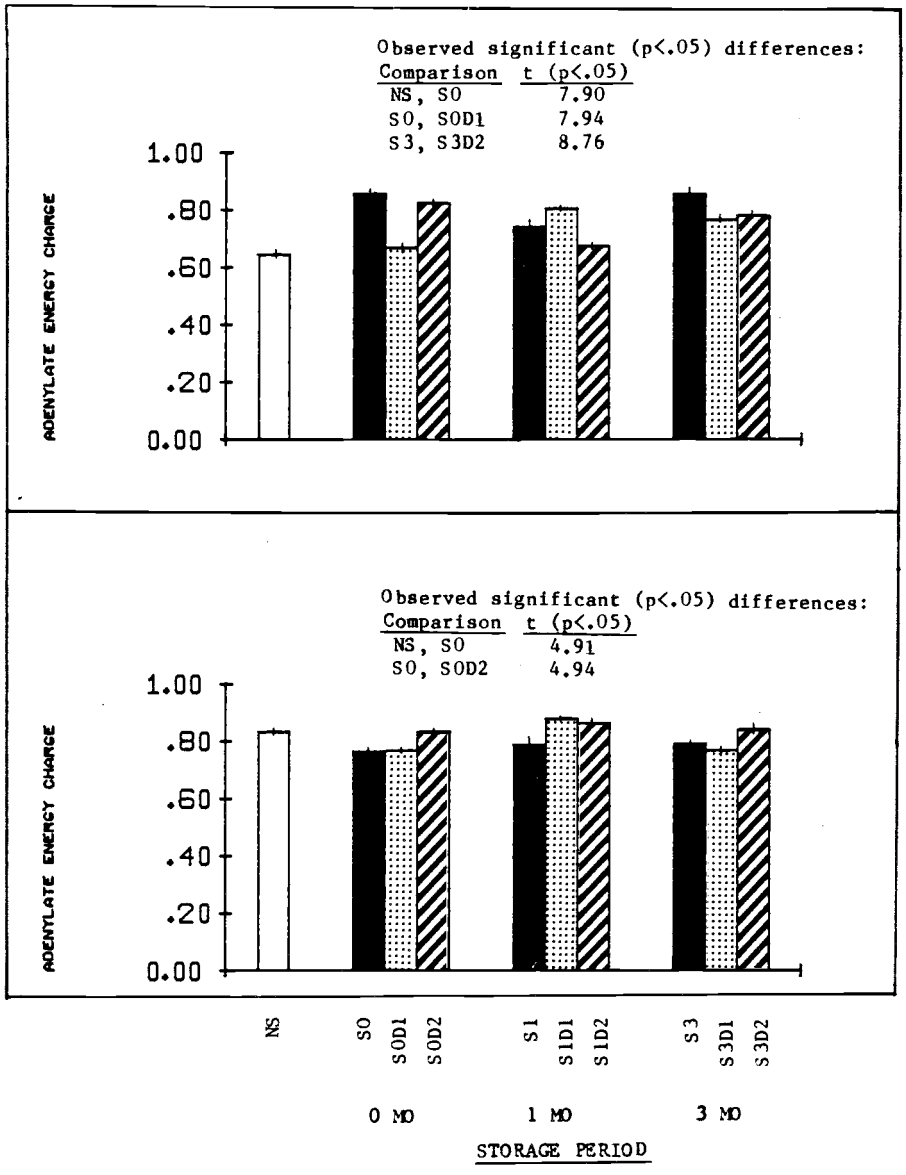
Figures III.35 and III.36. Effect of storage period and moisture content on ADP levels of 5-day-old seedlings originating from stratified Pseudotsuga menziesii seeds from zones 061 (Fig. III.35) and 252 (Fig. III.36). ■ - 45% moisture content, ⁞⁞⁞ - 35% moisture content, ▨ - 25% moisture content. Bars represent mean ADP levels of 3 replications of 4 seedlings. Error bars represent standard errors.



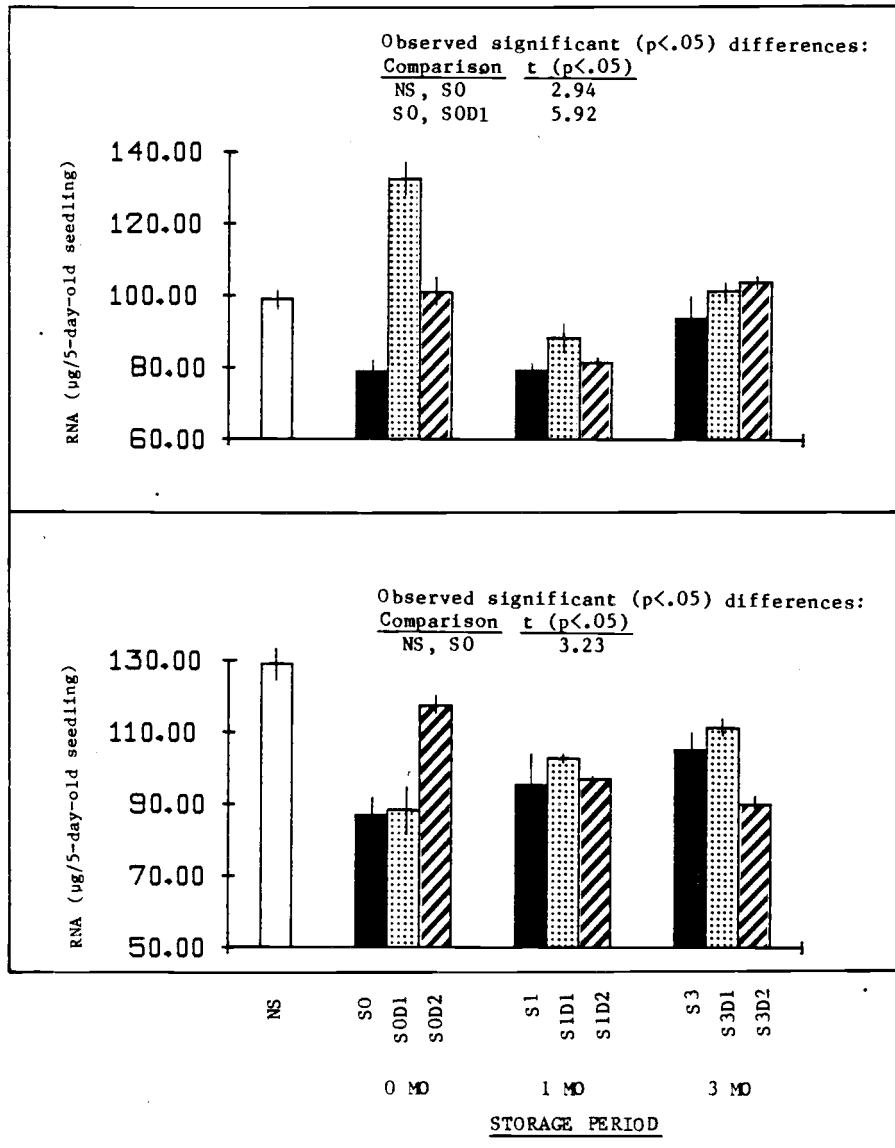
Figures III.37 and III.38. Effect of storage period and moisture content on AMP levels of 5-day-old seedlings originating from stratified Pseudotsuga menziesii seeds from zones 061 (Fig. III.37) and 252 (Fig. III.38). ■ - 45% moisture content, ⁞⁞⁞ - 35% moisture content, ▨ - 25% moisture content. Bars represent mean AMP levels of 3 replications of 4 seedlings. Error bars represent standard errors.



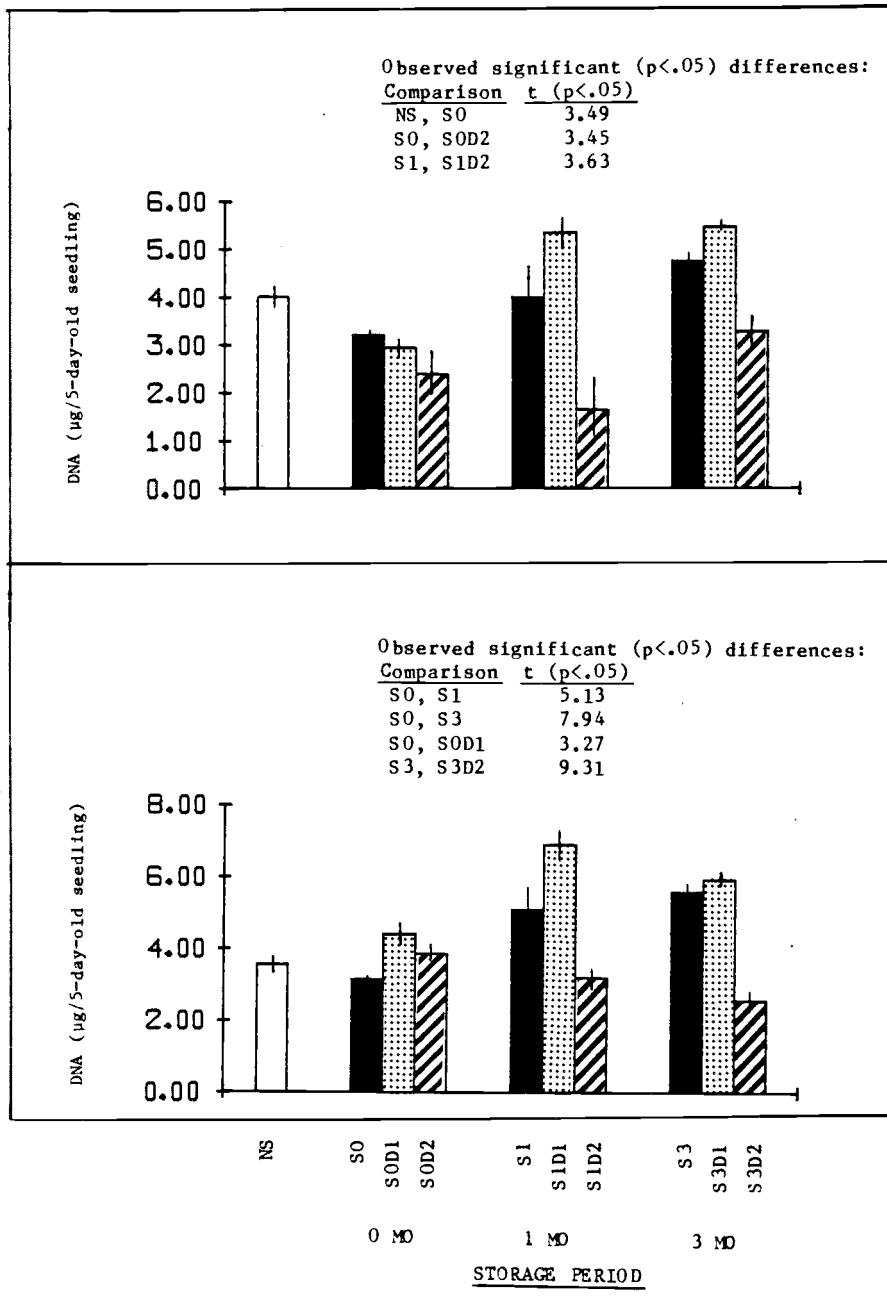
Figures III.39 and III.40. Effect of storage period and moisture content on TAP levels of 5-day-old seedlings originating from stratified Pseudotsuga menziesii seeds from zones 061 (Fig. III.39) and 252 (Fig. III.40). ■ - 45% moisture content, ⁞⁞⁞ - 35% moisture content, ▨ - 25% moisture content. Bars represent mean TAP levels of 3 replications of 4 seedlings. Error bars represent standard errors.



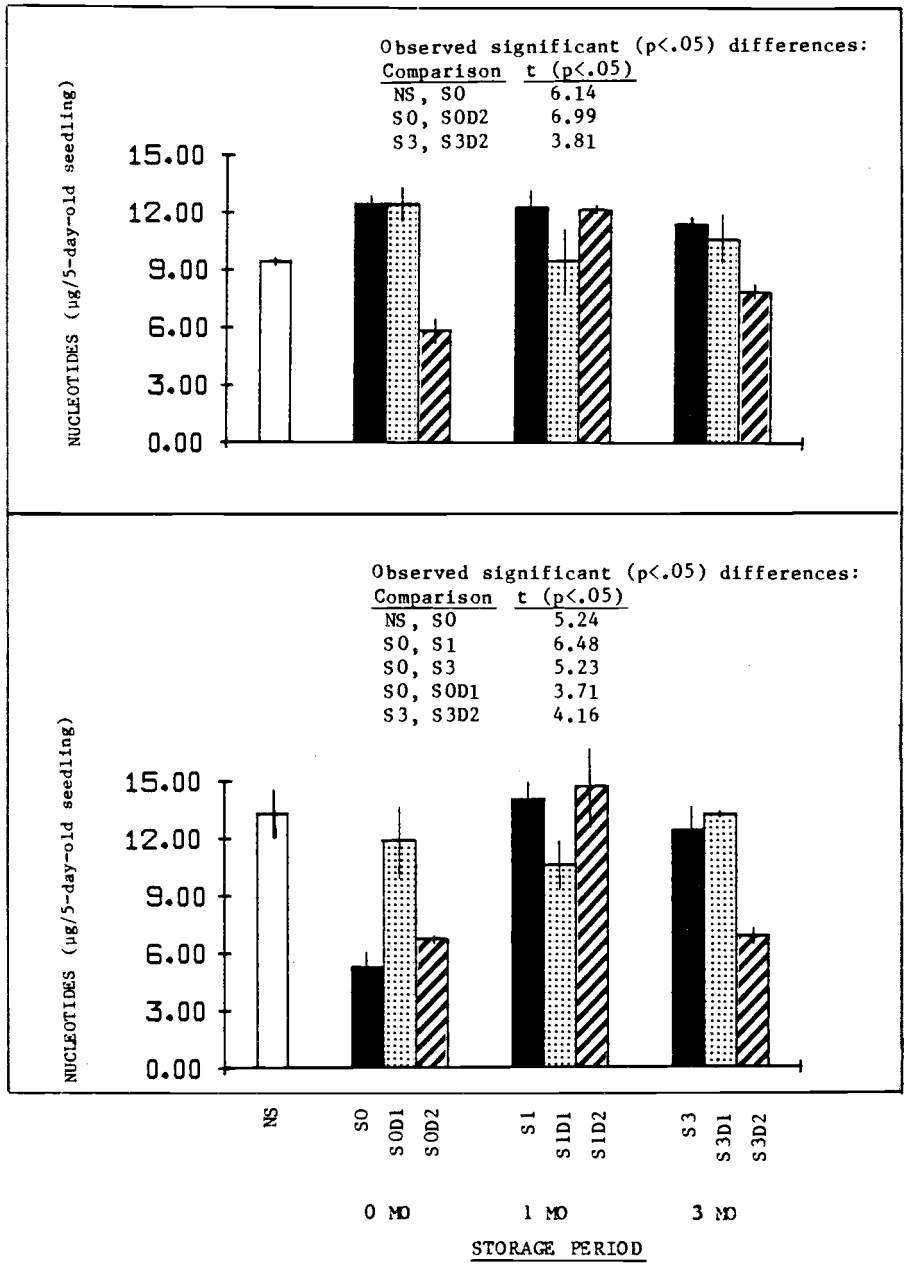
Figures III.41 and III.42. Effect of storage period and moisture content on adenylate energy charge of 5-day-old seedlings originating from stratified Pseudotsuga menziesii seeds from zones 061 (Fig. III.41) and 252 (Fig. III.42). ■ - 45% moisture content, ⁞⁞⁞ - 35% moisture content, ▨ - 25% moisture content. Bars represent mean adenylate energy charge of 3 replications of 4 seedlings. Error bars represent standard errors.



Figures III.43 and III.44. Effect of storage period and moisture content on RNA levels of 5-day-old seedlings originating from stratified Pseudotsuga menziesii seeds from zones 061 (Fig. III.43) and 252 (Fig. III.44). ■ - 45% moisture content, ⁞⁞⁞ - 35% moisture content, ▨ - 25% moisture content. Bars represent mean RNA levels of 3 replications of 4 seedlings. Error bars represent standard errors.



Figures III.45 and III.46. Effect of storage period and moisture content on DNA levels of 5-day-old seedlings originating from stratified Pseudotsuga menziesii seeds from zones 061 (Fig. III.45) and 252 (Fig. III.46). ■ - 45% moisture content, ⁞⁞⁞ - 35% moisture content, ▨ - 25% moisture content. Bars represent mean DNA levels of 3 replications of 4 seedlings. Error bars represent standard errors.



Figures III.47 and III.48. Effect of storage period and moisture content on nucleotides levels of 5-day-old seedlings originating from stratified Pseudotsuga menziesii seeds from zones 061 (Fig. III.47) and 252 (Fig. III.48). ■ - 45% moisture content, ⁞⁞⁞ - 35% moisture content, ▨ - 25% moisture content. Bars represent mean nucleotides levels of 3 replications of 4 seedlings. Error bars represent standard errors.

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CHAPTER IV. MOISTURE CONTENT OF VARIOUS STRUCTURES OF DOUGLAS-FIR SEEDS DURING STRATIFICATION, REDRYING AND STORAGE

Introduction

Seeds of Douglas-fir [Pseudotsuga menziesii (Mirb.) Franco] have a dormant embryo unless they are stratified or prechilled. Stratification is understood as the storage of seeds in the imbibed state, usually at low temperature for a certain time depending on the species. Studies showed that air-drying immediately following stratification is a method which maybe used to safely store conifer seeds remaining after sowing and prevent losses due to extended stratification period (Danielson and Tanaka 1978, Edwards 1981). Results reported by Edwards (1981) and in Chapters II and III suggest that not only can stratified Abies and Douglas-fir seeds be air-dried without losing stratification benefits but that moisture reduction might also promote improvement of seed metabolic processes and the production of heavier and larger seedlings than stratification alone. Because of the potentially important role of seed moisture content in this process, and lack of information regarding distribution of water among seed structures, the study reported here was initiated.

Materials and Methods

Two Douglas-fir seed lots with high germinative capacity were used in this study. Seeds from the 1980 collection were obtained from a commercial seed company. One lot was collected at the coastal seed zone 061 (elevation 0-500 ft) and the second at the interior seed zone 252 (elevation 501-1000 ft). Seeds were stored in airtight containers

at 1°C until used. Before experimentation began seeds were screened to obtain large and uniform size. Seed of both sources contained an average of 7% moisture.

Seeds were soaked in water at room temperature for 24 hr, drained, placed inside 4 mil polyethylene bags, then stratified at 3°C for 28 days. The moisture content of these stratified seeds then was adjusted to two lower levels: 25 percent and 35 percent. To reach the desired moisture content seeds were spread in a single layer over a mesh screen inside a standard room (temperature 21°C, RH 70 percent) for 20 minutes to 35 percent and 48 hours to 25 percent. Air dried seeds were placed in dry 4 mil polyethylene bags and returned to cold storage at 3°C for 3 months. Non-dried, stratified seeds were also stored. Their average moisture content was 45 percent for both seed lots.

Total seed moisture content was calculated after drying 4 samples of 10 seeds each for 24 hours at 105°C and expressed as a percentage of seed fresh weight by the formula:

$$\text{moisture content } \% = \frac{\text{fresh weight - dry weight}}{\text{fresh weight}} \times 100$$

To attain the target moisture contents, 10 samples of 10 seeds each from each lot were air dried inside the standard room for the following intervals: (1) every 5 minutes up to 1 hour, (2) every 1/2 hour up to 2 hours, (3) every hour up to 12 hours and (4) every 12 hours up to 48 hours. The mean moisture content was calculated by the oven dry method as described above. These means were used to decide

the duration of the air drying process in order to reach the desired moisture contents (35 percent and 25 percent).

The moisture content of seed parts was determined by dissecting 4 replications of 10 stratified seeds of each treatment inside a cold room (°C) with a RH% of 90. Nonstratified seeds were dissected inside a hot room (33°C) with a RH% of 32. The percentage of moisture was determined by the oven dry method and expressed as a percentage of fresh weight as described before.

Statistical Analysis

Analysis of variance by complete randomized design was conducted on all data. T-tests were used to determine differences (1) between nonstratified and stratified treatments, (2) among redrying treatments, and (3) between nonstored and stored treatments of each moisture level.

The following results were obtained by pooling data of the two sources because previous statistical analysis indicated no difference between seed sources.

The 5 percent probability level ($p < 0.05$) is used to test for statistical significance.

Results and Discussion

Each of the seed component parts hydrate to different extent during the stratification process. Table IV.1 gives the average percent moisture of nonstratified and stratified seeds and seed parts. Percentage of moisture increased by 7, 13, 11 and 6-fold in whole

seed, seed coat, embryo and gametophyte respectively. Similarly, Stiles (1948) found that the embryos of maize (Zea mays L.) and cotton (Gossipium hirsutum L.) took up water to a far greater extent than the other seed structures, and with the endosperm in maize and the cotyledons in cotton possibly acting as a reservoir of water for the embryo.

Results in our studies indicate that by redrying stratified seeds (45% moisture content) to 35% moisture content the embryo and gametophyte moisture content remained unchanged right after drying (Table IV.2). Seed coat moisture content was significantly reduced right after the process. Readjustment of moisture content could occur but was not determined in this study. Possibly the seed coat dynamic response to 20 minutes of air drying is due to the more external position of this tissue in relation to the other seed components. Also, it is known that the seed coat serves as transporting agent for water for the internal parts of seed (Stiles 1948). On the other hand, redrying stratified seed (45% moisture content) to 25% moisture content significantly reduced % of moisture in all seed structures (Table IV.2). This response might be explained by the fact that in order to reach the desired moisture seeds were exposed to the dry air for 48 hours. Thus, part of the embryo and gametophyte moisture had the chance to migrate towards the seed coat and evaporate from the seed surface to the surrounding air.

Generally, three months of storage did not affect moisture content of either seed structures or seeds of all moisture levels (Tables IV.3, IV.4 and IV.5). The exception was an apparent reduction in seed coat moisture content in stratified seeds after 3 months of storage (Table IV.3). However, Stiles (1948) found that in cotton and corn

seeds moisture within the seed is redistributed after 60 days. In addition, unlike Stone's (1957) findings our data did not indicate moisture loss in seeds or seed parts of the low vigor and low viability seeds from the three months of storage treatments (Chapter II). Perhaps the varying responses of Stiles, Stone and this study can be attributed to the different species used by workers.

Conclusions

Based on data reported in Chapters II and III it is suggested that moisture reduction of stratified seeds to both 35 and 25% appears to be an effective physiological mean to improve embryo and gametophyte metabolic activities and seed performance in laboratory conditions. If it is assumed that the moisture reduction was solely responsible for these responses the data reported here suggest that a very subtle variation in the seed parts' moisture content was enough to affect the inter-relationship between embryo and its surroundings and seed response to external stimuli. Possibly this subtle change in moisture content in stratified seeds redried to 35% moisture content could not be detected by our technique. However, we know very little of the events that lead to the results here reported. More research is needed to understand whether the moisture levels of seed parts or a combination of specific moisture content and a temperature sensitive mechanism triggered the observed enhancement of physiological and biochemical stratification benefits of stratified and redried seeds.

Table IV.1. Percent of moisture (W.W.B.) in nonstratified and stratified seeds and each seed part (M ± SE).

		Parts of seed		
Treatment	Total	Seed Coat	Embryo	Gametophyte
Nonstratified	6.0a ± .3	4.2a ± .2	4.7a ± .4	5.7a ± .3
Stratified	45.7b ± .5	55.5b ± .8	51.8b ± 1.6	35.0b ± 2.4

W.W.B. - WET WEIGHT BASIS

M - mean of 8 replications of 10 seeds.

SE - standard error

Column means with similar subscripts do not differ ($P > .05$).

Table IV.2 Percent of moisture (W.W.B.) in stratified and stratified redried seeds and each seed part (M ± SE).

Treatment	Total	Parts of seed		
		Seed Coat	Embryo	Gametophyte
Stratified (45% M.C.)	45.7a ± .5	55.5a ± .8	51.8a ± 1.6	35.0a ± 2.4
Stratified and redried to 35% M.C.	35.8b ± .7	31.2b ± 2.4	51.7a ± 2.4	35.7a ± .9
Stratified and redried to 25% M.C.	23.8c ± .7	18.7c ± .8	32.3b ± 2.0	22.6b ± 1.0

W.W.B. - WET WEIGHT BASIS

M - mean of 8 replications of 10 seeds.

SE - standard error

M.C. - moisture content

Column means with similar subscripts do not differ ($P > .05$).

Table IV.3. Percent of moisture (W.W.B.) in stratified seeds and each seed part after 0 and 3 months of storage (M ± SE).

Seed Part	Storage period	
	0 month	3 months
Total	45.7a ± .5	46.8a ± .7
Seed coat	55.5a ± .8	50.6b ± .8
Embryo	51.8a ± 1.6	50.3a ± 2.5
Gametophyte	35.0a ± 2.4	36.6a ± .4

W.W.B. - WET WEIGHT BASIS

M - mean of 8 replications of 10 seeds.

SE - standard error

M.C. - moisture content

Row means with similar subscripts do not differ ($p > .05$).

Table IV.4. Percent of moisture (W.W.B.) in stratified and redried to 35% M.C. seeds and each seed part after 0 and 3 months of storage (M ± SE).

Seed Part	Storage period	
	0 month	3 months
Total	35.8a ± .7	35.2a ± .3
Seed coat	31.2a ± 2.4	27.1a ± 1.2
Embryo	51.7a ± 2.4	47.6a ± 2.5
Gametophyte	35.7a ± .9	35.1a ± .7

W.W.B. - WET WEIGHT BASIS

M - mean of 8 replications of 10 seeds.

SE - standard error

M.C. - moisture content

Row means with similar subscripts do not differ ($p > .05$).

Table IV.5. Percent of moisture (W.W.B.) in stratified and redried to 25% M.C. seeds and each seed part after 0 and 3 months of storage (M ± SE).

Seed Part	Storage period	
	0 month	3 months
Total	23.8a ± .7	23.9a ± .3
Seed coat	18.7a ± .8	21.0a ± 1.6
Embryo	32.3a ± 2.0	30.6a ± 2.0
Gametophyte	22.6a ± 1.0	21.5a ± 2.7

W.W.B. - WET WEIGHT BASIS

M - mean of 8 replications of 10 seeds.

SE - standard error

M.C. - moisture content

Row means with similar subscripts do not differ ($p > .05$).

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CHAPTER V. GENERAL CONCLUSIONS

Results in Chapters II and III suggest that stratified seeds contained high phosphorylative efficiency and high RNA levels correlating with fast seed germination. Investigations into the stability of these physiological and biochemical benefits of stratification in relation to subsequent redrying and storage indicate that redrying seems to shift both the rate of nucleic acids synthesis and the rate of germination in parallel fashion. In addition, there is the production of vigorous seedlings. Therefore the data suggest a possible casual relationship between these biochemical and physiological processes. However, more detailed studies should be conducted in exploring the mechanism(s) of partial dehydration of hydrated tissues resulting in enhanced synthesis of protein, RNA and DNA at the early germination stages of seeds. ATP, total adenosine phosphates and energy charge levels of stratified seeds were stable during redrying. Possibly the steady state of energy observed was due to the rapid turnover of cell energy and a major demand of ATP for nucleic acid and nucleotides synthesis. In contrast with other reports these studies indicate that storage of stratified seeds for 1 and 3 months markedly lowered RNA levels of embryos and gametophytes indicating an enhanced RNase activity during the low temperature storage. There was a parallel reduction of energy status, RNA synthetic ability, seed and seedling vigor in stratified seeds at low temperature storage. Generally the loss of benefits of stratification and subsequent deterioration throughout storage were similar for stratified and stratifed and redried seeds. None of the biochemical criteria studied in

the 5-day-old seedling showed close proportionality with either physiological responses or the biochemical changes obtained in the seed stage. Possibly seedlings' morphological and biochemical development were not in pace among different treatments resulting in varied quantity of biochemicals. Perhaps older seedlings will express the stratification, redrying and storage effects in more explicit fashion.

Data in Chapter IV indicate that each of the several tissues making up the seed hydrate from an average of 6% to varied extent after stratification. Stratified seeds contained 46, 56, 52 and 32% moisture content in whole seed, seed coat, embryo and gametophyte, respectively. Moisture reduction of stratified seeds to 35% did not change embryo and gametophyte moisture. On the other hand, redrying stratified seeds to 25% significantly reduced % of moisture in all seed structures. However, based on data reported in Chapters II and III it is suggested that redrying stratified seeds to 35 and 25% is similarly effective in improving seed metabolic activities and performance, regardless of the varied moisture content of seed component parts. Also, data in Chapter IV indicate that generally three months of storage did not alter moisture content of either seed structures or seeds of all moisture levels. More research is needed to understand whether a subtle variation in seed parts moisture content or a combination of specific moisture content and a temperature-sensitive mechanism triggered the observed enhancement of physiological and biochemical stratification benefits of stratified and redried seeds.

These studies suggest that it would be advantageous to redry stratified seeds to a range of 25 to 35% before sowing to produce vigorous seedlings or to allow the expression of best benefits of

stratification. Further studies are needed to investigate the use of this technique on a production basis paying special attention to provenance, maturity and processing procedures.

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