Maturation of Noble Fir (*Abies procera*) and Grand Fir (*Abies grandis*) Seed

by

Jeffrey L. Snyder

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Completed December 1976

Commencement June 1977

AN ABSTRACT OF THE THESIS OF

JEFFREY L. SNYDER for the degree of MASTER OF SCIENCE

in FOREST SCIENCE presented on December 17, 1976

Title: MATURATION OF NOBLE FIR (Abies procera) AND GRAND FIR (Abies grandis) SEED

Abstract approved:

The objectives of this investigation were two-fold: (1) to examine possible seed and cone characteristics and their usefulness as seed maturity indices, and (2) to examine the effects of artificial ripening on seed maturation and germination. Cone fresh weight, cone length, cone specific gravity, cone color, seed fresh weight, seed development, seed wing color and embryo/embryo cavity ratio were studied to determine their usefulness as maturity indices. The artificial ripening treatments included: (1) detached cones stored outdoors in the shade, and (2) detached cones stored outdoors in the shade with their basal end setting in moist perlite.

With maturity, grand fir seed germination increased linearly without any leveling off prior to seed dispersal. Due to the erratic nature of noble fir seed germination no definitive pattern was observed. Artificial ripening techniques were beneficial for both species, increasing germination above that of the control and also

increasing the rate of germination. Seedling vigor, as measured by oven dry weight, was also improved by artificially ripening the immature cones. Dry outdoor storage of cones produced more total sound seed that moist outdoor storage.

The ratio of embryo length to the length of the embryo cavity was the single most useful index of seed maturity found. Seed wing color and degree of seed wing attachment also appear to be useful in assessing seed maturity. Cone color and cone specific gravity were beneficial cone characteristics found for determining seed maturity.

The most marked changes in biochemical constituents of ripening seed were the steady accumulation of reserves, i.e. crude fat and protein nitrogen, and structural components. Soluble sugar, amino acid and starch contents decreased with maturity, while crude fat and nitrogenous compounds increased. During artificial ripening an even more significant increase in crude fat and nitrogenous compounds occurred, thus reflecting a conversion from mobile to storage forms. The decrease in organic materials of cone scales also was associated with mobilization of these materials by maturing seeds. An even more significant decrease in these materials occurred after the cones had been picked and stored outdoors, suggesting translocation of substances from cone scales to ripening seeds.

ACKNOWLEDGEMENTS

I owe thanks to all these people involved in assisting me in the completion of this project: Tim Faytinger, Joyce Fatinger, Steve Cline, Steve Opdahl, Alan Doerkson, Ron Carlton, Kran Kilpatrick, Sandy Gordon, Ben and Lafgadio.

I would also like to thank Dr. Denis Lavender and Dr. Te May Ching for their assistance, guidance and review of this research.

Special thanks are due to Chris, my very best companion, whose patience and encouragement inspired me to complete this thesis. Her assistance and love were always there when they were needed.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDIX TABLES

MATURATION OF NOBLE FIR (Abies procera) AND GRAND FIR (Abies grandis) SEED

INTRODUCTION

The genus Abies is a northern hemisphere genus of about 40 evergreen tree species. Nine species of Abies are included in the coniferous flora of the United States; seven of these are scattered through the forests of the West. These species are not only valuable for timber but also are a valued source for pulpwood, ornamentals and Christmas trees. In addition, the true firs provide cover on mountainous sites that are critical to the maintenance of high-quality, well-regulated water supplies. And, these same areas are often important as recreation sites.

Due to the increased utilization of Abies species, particularly in the Pacific Northwest, there is a greater demand for regeneration of these stands. Modern reforestation has become more oriented toward nursery grown seedlings and less toward natural regeneration and aerial seeding. Consequently, an abundant supply of good quality seed is needed in order to grow the nursery seedlings.

However, much of the Abies seed found in the cone is empty, insect damaged, or blighted and the viability of the filled seed can be highly variable. Germinative capacity in a cleaned seedlot is typically poor, often averaging around 20 percent, creating a problem in

artificial regeneration as well as being a deterrent to successful regeneration in nature. Reasons for this have been ascribed as relating to pollination, maturation, collection, climate, insect infestation and damage during processing and storage of cones and seed (Ching, 1960; Franklin, 1965; Rediske and Nicholson, 1965; Pfister, 1967; Speers, 1969; Eis, 1973; Kulhavy and Schenk, 1976). The poor quality seed increases the cost of nursery stock.

The infrequency of good cone crops (3 to 7 years) combined with the low numbers of filled seed and their variability in germination, plus the rapid rate of seed deterioration even under very closely controlled storage conditions (Holmes and Buszewicz, 1958; USDA, 1974), magnify the problem of germination in Abies species for reforestation. Seed dormancy presents further difficulty and seedlots generally require a period of cold pretreatment to obtain maximum germination.

A systematic study of seed development of noble fir (Abies procera) and grand fir (Abies grandis) may indicate some methods of improving seed quality of true firs. Thus, this study was initiated with the following objectives:

1) to determine criteria of seed or cone characteristics which indicate maturity, and
2) to determine the effect of artificial ripening on seed maturation,

germinability and the accumulation of important biochemical constituents in ripening seed.

The degree of natural maturity and the effectiveness of artificial ripening methods were evaluated in terms of germination capacity and seedling vigor.

LITERATURE REVIEW

Flowering and Fruiting

The unisexual strobili of Abies are typically borne high in the crown of the tree. Female strobili are erect, solitary or in groups of two to four on the upper sides of last year's branchlets. Male strobili cluster densely along the undersides of one-year-old twigs and usually occupy a position lower in the crown than female strobili (Liu, 1971). The strobili of noble fir open from mid-May to mid-June and cones ripen in early September. The cones start to drop their scales and liberate the seeds in October of the same year leaving only the central cone spike attached to the branch. At maturity, cones are four to ten inches long, cylindrical, olive-brown, with tightly reflexed bracts covering the cone scales. Grand fir strobili also start to appear prior to vegetative bud burst, but due to the different habitat in which it is found this occurs from late April to mid-May. Cones mature in one season, are two to four and one-half inches long and range in color from yellowish green to greenish purple (Harrar and Harlow, 1969).

Mature Abies seed is ovoid to oblong and consists of a large wing, a thick rather hard seed coat (thin, soft coat in Abies grandis) with several resin vesicles on its surface and an embryo with five to ten cotyledons embedded in female gametophyte, a tissue with reserve food providing nutrition for the embryo. A full grown fir embryo

consists of an upper part differentiated into the cotyledons and the lower part into the hypocotyl, which terminates in a radicle.

Cone bearing may begin on 20- to 30-year-old trees and production increases with age. Good cone crops are usually produced at intervals of from two to four years, with light crops during intervening years (Franklin, 1968; USDA, 1974).

Physiological Development

During seed maturation there is a steady inflow of soluble compounds, such as simple sugars, amino acids and small amounts of inorganic substances, which are transported from other parts of the plant. From these substances the storage compounds of high molecular weight are gradually formed. Glucose is converted into starch and fats. Amino acids are required for protein synthesis. The whole process of seed maturation is accomplished by a coordinated pattern of enzyme systems. Reserve substances of ripe conifer seed are stored in the female gametophyte and to some extent in the embryo (Sarvas, 1965).

The physical and physiological changes occurring during cone and seed development in Douglas-fir were investigated by Ching and Ching (1962). They found that the moisture content and specific gravity of the cones decreased with maturity, while weight of cone, length of embryo, weight of seed and seedling vigor increased with

maturity. During seed development the starch was the major substrate provided by the cone scale and it was shifted to the embryo and female gametophyte as the seed matured.

Rediske (1961) in an earlier study examined the biochemical changes associated with maturation of Douglas-fir seed. Reducing sugar and non-reducing sugar content decreased, crude fat content increased, and the iodine number of this fat decreased as maturity was approached indicating an increase of saturated fat with seed maturity. Differences in starch, protein nitrogen and soluble nitrogen concentrations were relatively minor.

With noble fir, a general trend of decreasing reducing sugar concentration was observed as seed matured. Non-reducing sugars also decreased but the trend was not consistent. Soluble nitrogen and starch concentrations decreased with maturity. Crude fat content increased during this time while the iodine number of this fat fluctuated. A period of after-ripening was required in the cone after the accumulation of organic materials had been completed in order to produce viable seed (Rediske and Nicholson, 1965).

Nitrogenous substances are also rapidly translocated to ripening pine seeds during the embryo development period. The occurrence and increase of the viability of unripe seeds were accompanied with the accumulation of these nitrogenous reserves in Pinus thunbergii seed (Katsuta, 1961). Formation of protein in later stages of seed

development is considered to have close relation to the rapid increase of the germinative activity of ripe seeds.

Dickmann and Kozlowski (1969) also have shown this to be the case for Pinus resinosa seed. At the same time that mature strobili were beginning to senesce, nitrogen accumulated in high concentrations in the seed. Senescing cone tissue appeared to supply much of the nitrogen, phosphorus and potassium translocated into seeds. With maturity, starch grains were reduced in size and number in the scale but began to increase in the embryo, indicating a movement of carbohydrates into the developing seed.

Lipids are the main food reserve in most coniferous tree seeds. In pines, lipid reserves increase rapidly in the developing seed, first appearing in the gametophyte, then in the embryo (Konar, 1958). This is also true for mature noble fir seed (Rediske and Nicholson, 1965), Douglas-fir seed (Rediske, 1961; Ching and Ching, 1962) and other coniferous seed (Bennett, 1966; Hansen and Boderick, 1968). During seed maturation, the most important biochemical process is the change from mobile to storage forms of food within the embryo and female gametophyte.

Artificial Ripening

It is difficult to collect cones with identical and full maturity because of the large forest areas and different environmental

conditions. If artificial ripening methods could be developed, then fully matured cones could be obtained for seed extraction.

Silen (1958), impressed with the possibility that squirrels might provide conditions for ripening immature cones in their storage methods, simulated squirrel caches and found that Douglas-fir cones collected early and stored in sandy soil had an appreciable percentage of germinable seed. He then collected cones beginning in July and continuing to natural maturity and stored them under three conditions: (1) dry storage, (2) damp storage in peat moss and (3) wet storage in water. Cones remained in storage until the end of September at which time germination tests were begun. Seed from cones in dry storage did not germinate from any collections prior to August 20, whereas seed from cones in damp storage showed appreciable germination from collections on August 1.

Similar results were obtained by Krugman (1966) using sugar pine seeds. Immature sugar pine seeds were collected and ripened either in the cone or in moist vermiculite. Seeds collected after the second week of August could be brought to maturity in the cones. It was suggested that for these artificial ripening techniques to succeed, the immature seeds must reach a stage of physiological and anatomical maturity at which the seeds are no longer dependent directly on the tree for nutrition. The reserves in the cone scales are apparently sufficient for seed development.

Rediske (1961) found that storage of immature Douglas-fir cones in burlap bags in the shade was beneficial to ripening the seed. Seeds containing 22 mg per gram of reducing sugar were immature (13 mg per gram indicated maturity), but could be ripened artificially. Results of this study point out that artificial ripening involves a complex of factors, some of which may serve as indicators of maturation. However, much more needs to be known about the content and state of enzymes and metabolites in seeds before it becomes clear what exactly occurs during this process. Only mature cones which were processed at picking time yielded the highest quality seed. Seeds of Abies have a dormancy problem which often requires a period of after-ripening. This was observed by Rediske and Nicholson (1965). They found that storage of immature and mature noble fir cones resulted in increased seed viability. Cones picked prior to maturity continued to provide mobile organic materials necessary for seed maturation as evidenced by increase in dry weight and endosperm density. At least four weeks of cone ripening were required for mature seed to reach maximum seed viability, indicating a period of after-ripening was necessary after the accumulation of organic materials had been completed. Edwards (1969) found similar results and suggested that immediate air drying and extraction of earlier collected cones is deleterious to germinability, because the rapid desiccation used in cone drying arrests the ripening process.

In an exploratory study of cone maturity in noble fir, Franklin (1965) collected cones at two week intervals and stored the cones as: (1) dry, (2) packed in paper wadding and (3) packed in moist peat moss. Storage of cones by the latter two artificial methods was not beneficial. Moist storage was the most detrimental, deterioration being attributed to mold. Franklin concluded that early collection of noble fir cones was not the primary cause of poor seed germination. Treatment of cones after collection, extraction and cleaning of seed and seed storage conditions may all contribute to a decreased germination of the seed.

Church and Sucoff (1960) reported that the viability of immature seeds of Pinus virginiana increased if the seeds were left in the cones of felled trees. Later, Fenton and Sucoff (1965) found that ripening and subsequent germination of Virginia pine seeds collected six weeks prior to seed dissemination and removed from the cone could be improved by prolonged cold dry storage in closed containers.

Artificial ripening of white fir seeds has also been successful (Oliver, 1974). Cones collected four weeks before seedfall and stored in a cool, moist environment for four weeks yielded seed which germinated as completely and rapidly as stratified seeds from mature cones. Storing immature white fir cones two weeks beyond beginning of seedfall boosted both speed and completeness of germination beyond that of stratified seeds from mature cones. This suggests

that white fir requires some after-ripening for maximum germinability.

Artificial ripening has been used successfully on immature Douglas-fir, noble fir, grand fir, white fir and several species of pine cones. The studies cited suggest that immature seeds of some conifers reach a stage where they no longer depend on the tree for further development. Storage of cones under conditions which favor movement of food materials into the seed, in order to attain maturity, appears practical as a means of extending the cone collection period. A possible reduction in quality due to fungi development under these conditions may be eliminated by proper aeration and mild fungicide treatment.

Maturity Indices

Cones should be collected when they are ripe and before their seeds have been dispersed. Collection of immature cones results in lower seed yield, inferior seed quality and abnormal germination of some species. Seed which is completely mature also stores better than immature seed (Eliason and Hill, 1954; Wakeley, 1954; Olson and Silen, 1975). Indices such as date of squirrel cutting, cone color, cone moisture content, seed firmness, specific gravity and embryo development have been tested on many coniferous species. Biochemical criteria of seed maturity also prove useful for some

selected species (Rediske, 1961; Rediske and Nicholson, 1965). These indices are used to assist collectors in harvesting the maximum possible seed crop at the correct time.

Physical indices of cone or seed ripeness are by far the most commonly used indicators. Browning of the cone bracts is recommended as a guide to cone collection for Douglas-fir (Ching and Ching, 1962). Color of seed wings appeared promising as a maturity index for grand fir (Pfister, 1967). A purpling in balsam fir cones suggests maturity (Stoeckeler and Jones, 1957). Among the pines, white pine seed is mature when the cones turn yellowish green with brown on the scale tips; and jack pine cones are ripe when half or more of the cone surface is brown (Stoeckeler and Jones, 1957). However, in some instances the variation between trees may be too great and color descriptions too difficult to make cone or seed color an effective index of maturity.

With experience, the firmness of the cone and seed can be a good indication of maturity. The time when cones become flexible and seed loses its milky character is indicative of maturation for many species (Oliver, 1974; USDA, 1974). This criterion as well as the previous one is subjective and cannot be used for defining specific stages of maturity.

Specific gravity is a more objective index of seed maturity which is based on water loss during maturation. Once the relationship

between seed maturity and specific gravity of freshly picked cones has been established, then a container of suitable liquid of a known specific gravity can be carried into the field for testing relative specific gravity of a sample of cones. Specific gravity has been used more widely than any other index for assessing maturity of coniferous cones. A substantial list of specific gravity indices for coniferous cones can be found in the Seeds of Woody Plants in the United States (Agricultural Handbook No. 450).

In general, decreasing moisture content accompanies maturation and its use as a maturity index has been suggested for balsam fir (Bakuzis and Hansen, 1965) and white spruce (Cram and Worden, 1957). This determination of maturity does require oven-drying and is not very suitable as a rapid field method for determining cone ripeness.

Ching and Ching (1962) found that embryo growth was a reliable index for Douglas-fir and that cones were mature when more than 90 percent of the embryo cavity was filled. The ratio of embryo length to the length of the embryo cavity was also the single most useful index of seed maturity for white fir and red fir (Oliver, 1974).

As the seed matures a number of measurable chemical changes take place. By correlating the relative amounts of selected biochemical constituents with seed maturity, a biochemical index of ripeness can be developed. For Douglas-fir, as an example, the reducing

sugar content was found to be a good indicator of maturity (Rediske, 1961), while for noble fir a crude fat content greater than 25 percent indicated that the seed would be viable if properly after-ripened (Rediske and Nicholson, 1965). Although biochemical analyses may give relatively precise estimates of seed maturity for research purposes, the method is not applicable to field determinations. However, when large collections are planned a knowledge of the correlation between biochemical constituents and seed maturity may be of value (USDA, 1974).

Lavender and Engstrom (1956) found that squirrels cut Douglas-fir cones in quantity only when the seeds are ripe, and the seeds demonstrated no significant increase in viability with later dates of cutting. Observations in the Pacific Northwest suggest squirrels do not cut in quantity until after Abies cones have matured, and the cones are typically cached in cool, moist sites conducive to after-ripening (Franklin, 1965). There is no evidence that seed collected in this way is inferior, consequently collection of squirrel cut cones has been used as a means of collecting mature seed even though seed quality may be quite variable.

MATERIALS AND METHODS

Cone Collection

Cones were collected during the summer and early autumn of 1975 from two different noble fir stands and one grand fir stand. The two trees in the first noble fir stand were located at the edge of a dominant noble fir stand on the west slope of Marys Peak in the Oregon Coast Range (elevation 1437 m). Also consisting of two trees, the other noble fir site was located in a mixed true fir stand on the southeast slope of Iron Mountain in the Oregon Cascades (elevation 1791 m). Tree ages were estimated as 75 to 80 years and 50 to 60 years respectively. Tree heights were estimated as 24 to 27 m (65 to 75 feet) for the former area and 16 to 20 m (45 to 55 feet) for the latter plot.

Since the cone crop for grand fir in the Willamette Valley and Coast Range was poor in 1975, difficulty in locating trees with a sufficient quantity of cones delayed collections until the first week in September. At that time two study trees were located in a mixed ponderosa pine-grand fir stand on the east side of the Oregon Cascades, five miles south of Suttle Lake (elevation 1211 m). As a result of this delay in collection of grand fir cones, additional collections were made during the summer of 1976. These trees were

estimated as 65 to 70 years old and had attained a height of 31 to 36 m (85 to 100 feet).

In choosing these study trees consideration was given to cone crop and ease of access and climability. Trees selected had full, deep crown development and carried cones over 20 percent of the crown height. Cones were cut or picked from all parts of the crown, in an effort to provide a representative sample at each collection date. Individual cones apparently damaged by insects, as evidenced by abnormal shapes and larvae emergence holes, were discarded.

Collection of cones was started in late July and ended when the cones had begun to disintegrate, sometime in October. Sampling intervals ranged from 12 to 18 days with a total of six sampling dates for each study area. In the case of noble fir, each collection comprised harvesting a total of 27 to 30 cones from both trees. Due to their smaller size a total of approximately 40 to 45 grand fir cones was harvested each time. The cones were immediately placed in polyethylene bags which were tied shut to prevent excessive moisture loss, and were packed in ice in a styrofoam chest for transport to the laboratory.

Cone Measurements and Treatments

Upon returning to the laboratory, ten cones were randomly selected and fresh weight, length and volume by water displacement

was determined. Volume of the cones was determined by water displacement so that their specific gravity could be calculated. Cones from each collection were then refrigerated overnight.

Following the overnight post harvest cold storage, the cones from each collection were randomly divided into three equal lots for treatment as follows:

1) immediate study without any treatment and regarded as fresh;
2) subjected toa period of outdoor storage on racks in a lathhouse and regarded as dry; and
3) subjected to a period of outdoor storage in a lathouse where detached cones were set with basal end in a plastic tray containing perlite, kept damp by watering every other day; once a week a nutrient solution was used.[1]

Half of the fresh cones were completely broken down by hand and seed and cone scale material was placed in separate glass containers. Observations were recorded on cone color, seed color and firmness of freshly picked cones. Fresh seed weight was determined for a random 100 seed sample. Twenty sound seeds were cut into longitudinal halves. Length of embryo and of embryo cavity in the gametophyte of the dissected seeds was measured with a micrometer

---

[1] Formulation for the modified Hoagland nutrient solution is described in Appendix Table 1.

under an Epi-illumination microscope (18x). Seed and cone scale samples were then refrigerated at -10 to -12°C until December, when chemical analyses began.

At each sampling time the remaining cones were broken up and dried at 34°C in a hot, dry air room. Upon reaching a low moisture content of six to ten percent, the seed was removed, dewinged by hand and stored in screw top glass containers at 0 to 2°C until spring of 1976, when germination tests were performed. This seed moisture content and storage temperature have been suggested for noble fir seed by Danielson (1974).

Germination and Seedling Vigor

Germination tests were conducted in covered plastic dishes measuring 12 by 12 by 3 cm. The dishes were partially filled with vermiculite which was uniformly moistened with tap water. One hundred seeds were then placed on top of the substrate in each dish. Each collection and artificial ripening cone treatment had four replications. The daily temperature regime was 20°C for 16 hours in the dark and 30°C for eight hours in the light. Another four dishes with seeds on moist vermiculite were stratified in the dark at 0 to 2°C for four weeks, then were incubated under the above conditions. Prior to the start of germination and stratification the seeds for each lot were soaked in 500 ml of distilled water for 48 hours at room

temperature, to allow the seed to imbibe water. The temperature used for germination of Abies seed has been suggested by the International Seed Testing Association (1966).

Germinated seeds were counted at intervals from two to five days for a 30 day period. A seed was counted as germinated when the radicle length was equal to the length of the seed. At the end of the test, a cutting test was performed on the remaining seeds. These seeds were classified as empty, insect damaged or filled. The filled seeds were tested with a one percent solution of tetrazolium chloride, as suggested by Danielson (1972), to determine which seeds were viable and capable of germinating.

The germination rate as used by Danielson (1974) and others was calculated for each seedlot of all collections and cone treatments described in the study. Germination rate is defined as the summation of the quotients produced by dividing the number of new germinants each week by the number of weeks at which the count was made. It may be expressed:

$$GR = \frac{\text{number of germinants}}{\text{week 1}} + \ldots + \frac{\text{number of germinants}}{\text{final week}}$$

Percentage of germination was calculated on the basis of total filled seed. Total filled seed was calculated as the number of seeds that germinated plus the tetrazolium stained seed.

The germinated seeds from each counting were grouped according to treatment and dates when the cones were collected, after each germination tally. The grouped germinated seeds were then planted, 30 seedlings per container, in a 25-cm-deep cardboard container, with a 25 cm diameter and filled with forest soil. After a period of 30 days with a constant temperature of 20 to 22°C and a 16 hour photoperiod of 400 foot-candles, seedlings were lifted and washed. Shoot and root lengths were measured individually and an average root to shoot ratio was calculated. After drying the seedlings for 24 hours at 70°C average dry weights were calculated by dividing by the number of individuals in the group.

Artificial Ripening

The stored cones were subjected to the two artificial-ripening treatments described previously. For storage in the lathhouse, trays were shelved under a table in the shade, where ambient air temperatures ranged from 9 to 32°C. When the cones in each lot began to open and fall apart, the storage treatment was deemed to have ended; the entire sample was removed and investigations began. This usually occurred within five weeks of the last collection date.

At the completion of each treatment the cones were completely broken down by hand. Seed and cone scale material was placed in separate glass containers and refrigerated at -10 to -12°C until

chemical analyses were begun. Twenty sound seeds were cut into longitudinal halves in order to measure the embryo to embryo cavity ratio as described earlier. The remaining seed material was dried to a low moisture content and refrigerated until germination and seedling vigor tests were performed.

Chemical Analyses

Both cone scale and seed materials were analyzed for free amino acids, soluble sugars, soluble nitrogenous compounds, insoluble nitrogenous compounds, starch, and crude fat content. A one gram sample of seed material, without seedcoat, was ground in 20 ml of 85 percent ethanol in a tissue grinder, filtered, and the above analyses performed.

For crude fat determination, a 14 ml aliquot of the ethanol extract was evaporated using a rotary vacuum evaporator. The residue was then dissolved in 30 ml of ether-petroleum ether (1:1) and agitated for five minutes. This solution was then evaporated at 45°C and the remaining residue dissolved in 20 ml of ether and transferred to a separatory funnel. Ten milliliters of petroleum ether was used to wash the flask and then the ether-petroleum ether mix was washed with 100 ml of distilled water. With the washing discarded, 5 ml aliquots of the ether-petroleum ether were transferred to small aluminum foil thimbles of known weight for complete drying at 70°C

for 20 hours. Weights of the residue in the thimbles represented estimates of crude fat.

The precipitant of the original ether-petroleum ether (1:1) extract was dissolved in distilled water. Soluble sugar and amino acid contents were analyzed on one ml aliquots according to the Anthrone Method (Yemme and Willis, 1954; Ching, 1975, personal communication) and the Modified Moore-Stein Method (Moore and Stein, 1954; Ching, 1975, personal communication) respectively.

Soluble nitrogen determination was carried out on 3 ml aliquots of the 85 percent ethanol extract. Each aliquot was placed in a 50 ml evaporation flask, dried and a micro-Kjeldahl determination was made on the residue (Doerkson and Carmichael, 1975, personal communication).

Protein nitrogen and starch analyses were made on the original residue of the 85 percent ethanol extract. Protein nitrogen was determined on 40 mg samples (Doerkson and Carmichael, 1975, personal communication). Starch was analyzed by the enzyme technique of Dekker and Richards (1971), as modified in our laboratory (Webb, 1976, personal communication).

Due to the hard nature of the cone scale material, a 2 g sample was ground to fineness with an Omni-mixer prior to analysis. This sample was extracted in 30 ml of 85 percent ethanol and analyses were performed on aliquots of this solution. Crude fat content was not

analyzed due to the abundance of resin in this material. Resinous materials plus crude fat would dissolve in petroleum ether and upon drying the residue that remained would not only contain crude fat, but other impurities as well.

Extraction of the cone scale material with 85 percent ethanol not only dissolved the soluble sugars, amino acids and soluble nitrogenous compounds, but also the pigments and compounds responsible for the purple color of the cone scales. Consequently, when the dried residue of this solution was dissolved in water a purple to red color appeared. These pigments then presented a unique problem by their interference with the colorimetric analysis of soluble sugar and amino acids. It has been shown elsewhere in the literature that conifer material presents unique problems in terms of interfering substances, particularly when colorimetric reactions are measured with a spectrophotometer (Ebell, 1968). In order to prevent an overestimation of soluble sugars and amino acids in cone scales, Polyclar AT[2] was used to clear the water extract of pigments responsible for the purple color in cone scales (Morris, 1976, personal communication). Three and one-half grams of hydrated Polyclar AT were added to the 10 ml water extract, agitated for ten minutes and centrifuged; aliquots were removed for sugar and amino acid examination as mentioned previously. This particular method allowed for 90 percent

---

[2]Polyvinylpolypyrrolidone is a product of GAF Corporation.

recovery of these compounds in their respective extracts (Appendix Table 3).

All chemical analyses were conducted in duplicate to agree within 15 percent of the mean. Results are expressed as milligrams of material per gram of fresh seed and cone scale weight.

RESULTS

Physical Characteristics of the Cone

Cone Characteristics

By the middle of September the noble fir cones had reached their mature size and the bracts were yellow green in color. The cones remained yellow green throughout September and into October before turning light brown to brown by the last collection date.

Grand fir cones attained mature size by September and were yellow green to olive green. At the beginning of seedfall all cones were yellow green to brown. The variation between trees was fairly significant however, and color descriptions of cones would probably not be a single, effective index of maturity in most cases. Table 1 lists the various changes in cone and seed characteristics which occurred over the time period of cone collections.

Cone Weight and Cone Length

The measurements presented in Figure 1 display the rapid losses in cone weight observed during the collection period for noble fir. Weight losses, largely due to removal of moisture, continued at a more or less uniform pace in cones from all stands during the last four weeks of collection. Figure 2 shows that noble fir cone length

Table 1. Sequence of changes in noble fir and grand fir cone and seed characteristics.

Date of collection	Seed wing color	Cone scale attachment	Megagametophyte consistency	Cone appearance	Seed development
			Grand fir		
July 28	deep red	firm	milky	green	white seed-firmly attached to cone scale
August 10	magenta	firm	milky	green	white seed turning green; early embryo stage with suspensor cells
August 30	magenta	firm	firm	olive-green	light green seed; cotyledons developing
September 3	magenta	firm	firm	olive-green to yellow-green	green seed; embryo with 6 or 7 cotyledons
September 18	red-brown	loose to firm	firm	yellow-green	seed turning brown; fully developed embryo
September 29	brown	loose	firm	yellow-green to brown	developed embryo
			Noble fir		
July 31	deep red	firm	milky	green bracts	white seed-firmly attached to cone scale; embryo transparent
August 12	magenta	firm	milky	green bracts	"
August 25	magenta	firm	milky	bracts turning purple	early embryo with suspensor cells
September 7	magenta	firm	firm	green-purple bracts	yellow seed; cotyledons developing around shoot apex
September 23	magenta-brown streaks	loose	firm	bracts are browning	fully developed embryo with 5 or 6 cotyledons
October 11	brown	loose	firm	yellow-brown bracts	light brown seeds

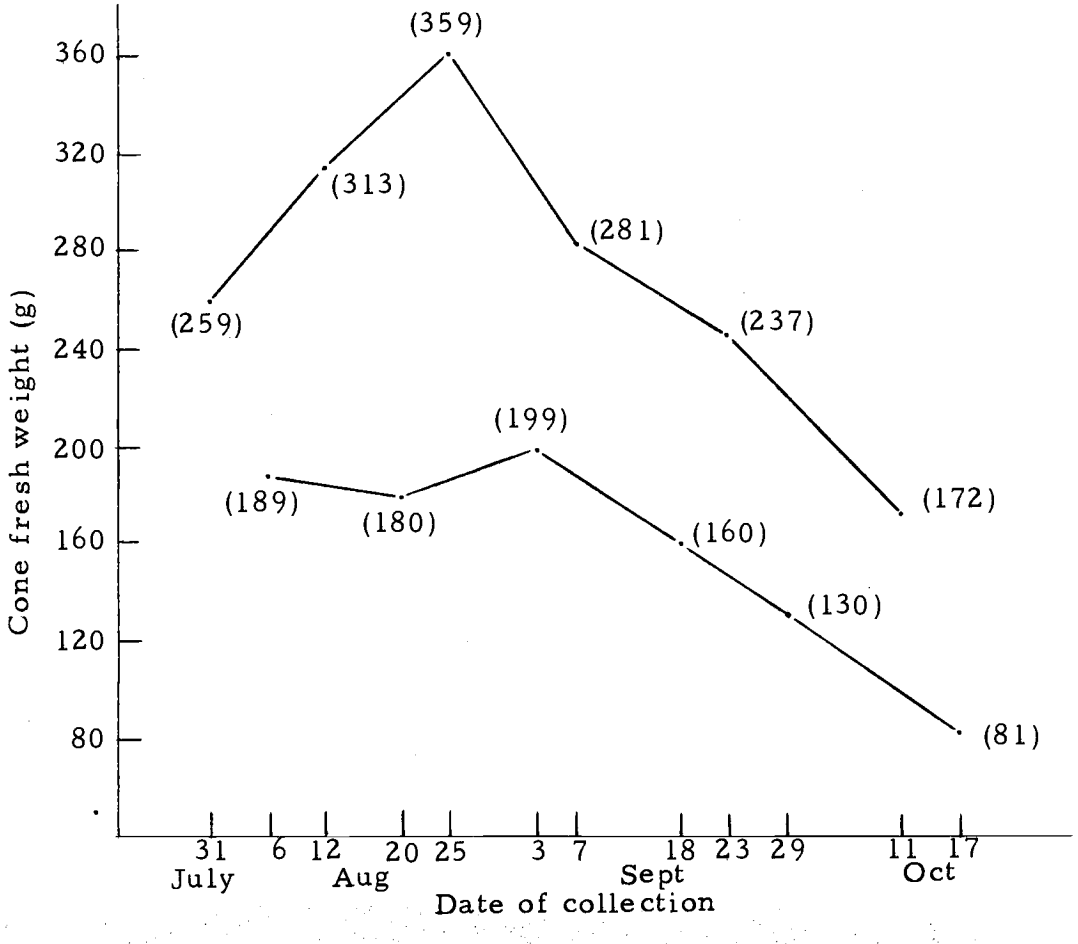


Figure 1. Noble fir cone fresh weight.

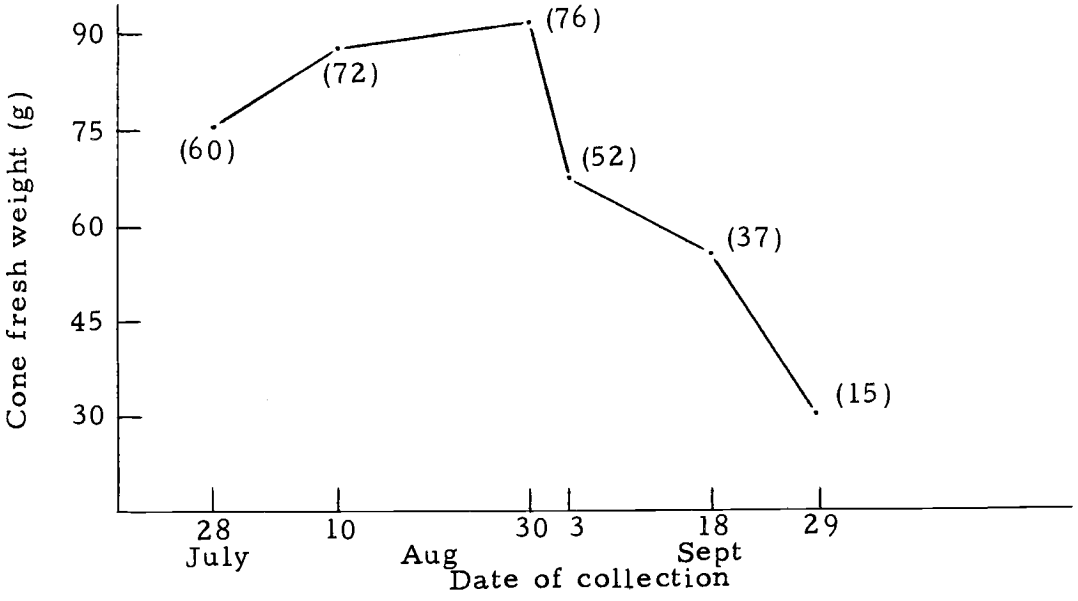


Figure 1A. Grand fir cone fresh weight.

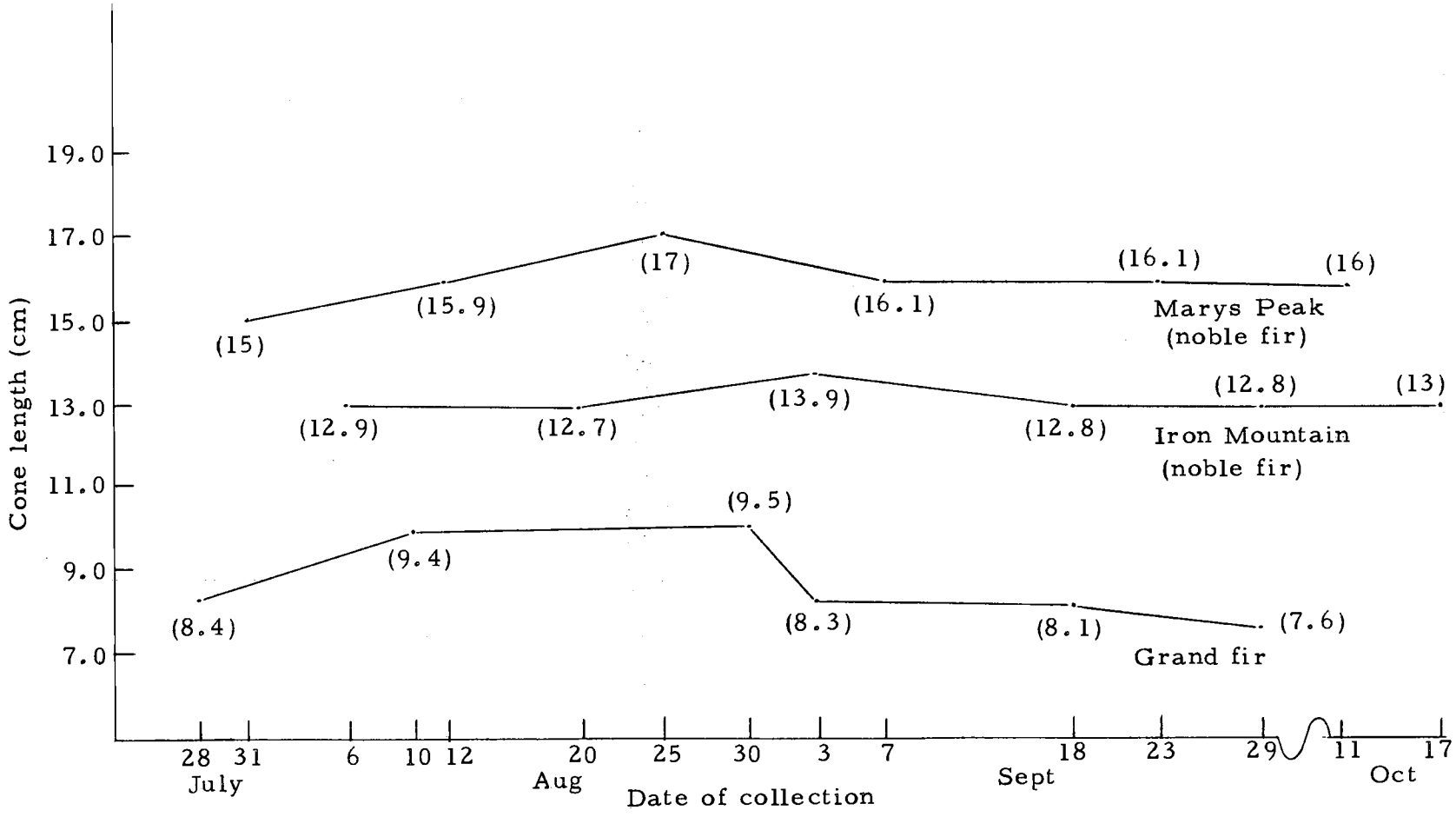


Figure 2. Cone length: grand fir and noble fir.

tended to increase during the collection period; however, the fluctuations recorded appear to be of little value as a parameter of cone maturity.

The fresh cone weight of grand fir displayed an even more rapid loss than did noble fir cone weight as maturity approached (Figure 1A). However, Figure 2 illustrates that there was a slight decrease in the cone length of grand fir during the collection period. This may be attributable to the fact that collections were made during two different growing seasons.

Cone Specific Gravity

Alteration of specific gravity, as shown in Figure 3 for all stands, closely parallels the losses in cone weight. Decreasing specific gravity was recorded throughout the collection period. After September 1 the decrease in specific gravity was quite uniform for both species and reached an observed low at the last collection dates. Specific gravity was correlated with percent germination of sound seed ($r = 0.88$).

Seed Characteristics

At the first collection date for both species, seeds were firmly attached to their cone scales, the female megagametophyte was transparent and had a milky consistency, the embryo was extremely small

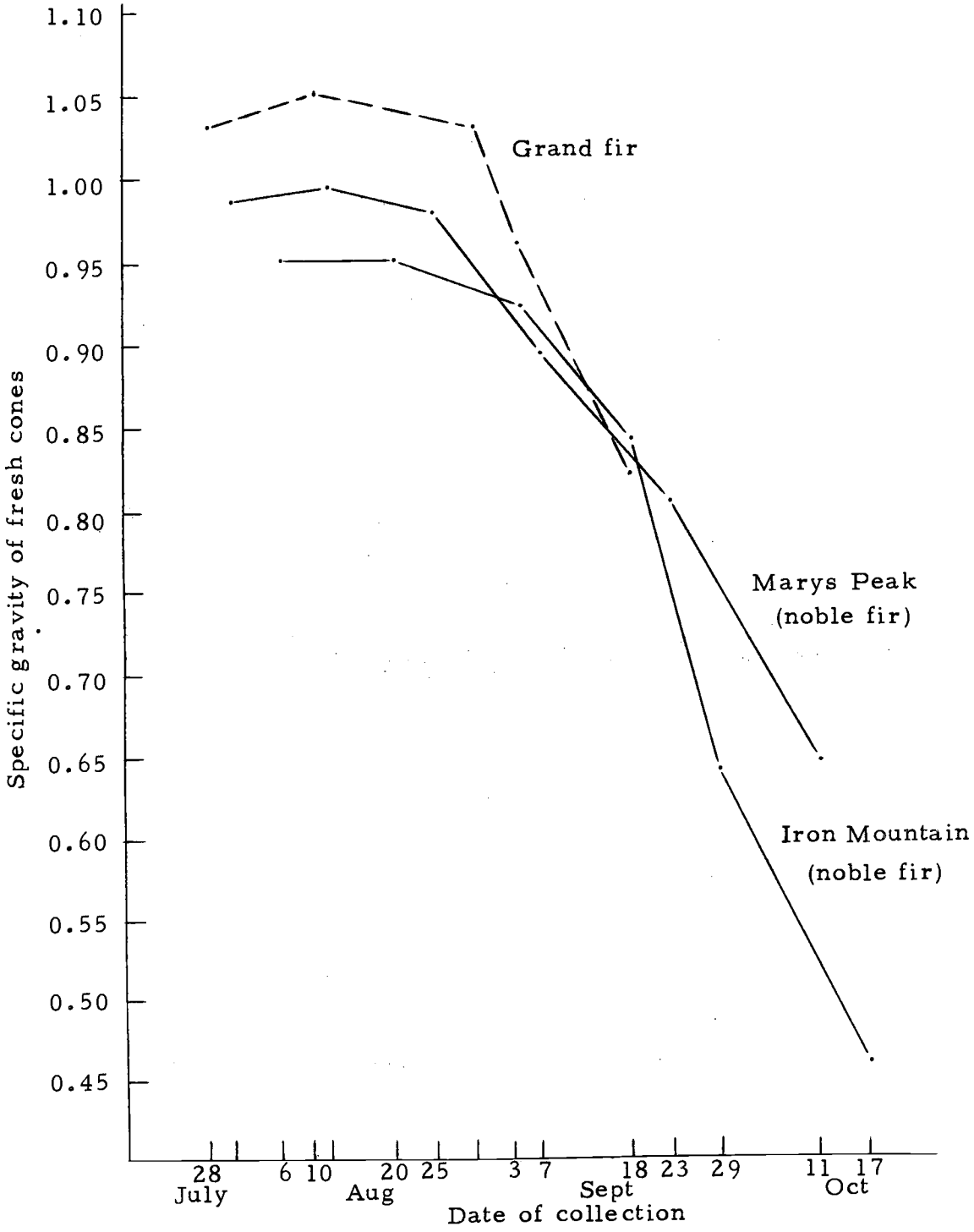


Figure 3. Specific gravity of fresh cones: grand fir and noble fir (Appendix Table 4).

and the seed wings were dark red in color. By late August the seed wings were magenta and were not attached to the cone scale and the embryo occupied over half the length of its cavity in the megagametophyte. Suspensor cells were beginning to disappear and the cotyledons were beginning to develop around the shoot apex. A tinge of yellow was now noticeable on the once green seed. By the last collection date the seed wing and seed were light brown to brown in color. The megagametophyte was firm and white in color and contained a fully developed yellow green embryo.

Seed Fresh Weight

In the case of grand fir seed a decreasing trend of seed weight was observed during the study period (Figure 4). A similar trend, although more gradual, that was noticed was the fresh seed weight from the noble fir stand in the Cascade Mountains. However, somewhat larger fluctuations in noble fir seed weight were measured from seed collected in the Coast Range. There would appear to be no value in these observations as a ripeness index.

Embryo to Embryo Cavity Ratio

Embryo to embryo cavity ratio showed a rapid increase during the study period, as seen in Figure 5. By three weeks prior to the last collection date, embryo length had attained a maximum value in

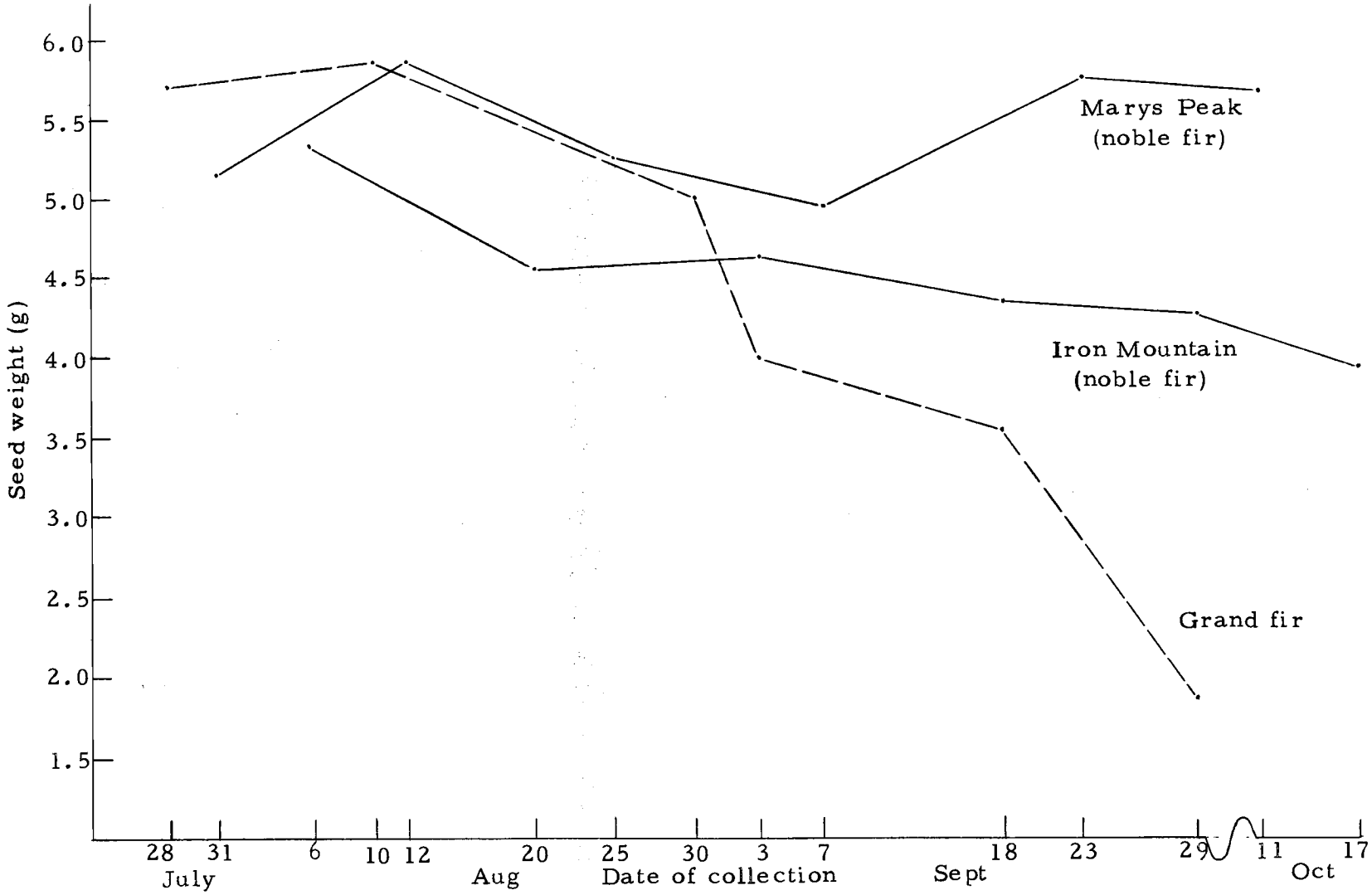


Figure 4. Fresh seed weight of 100 seeds (Appendix Table 4).

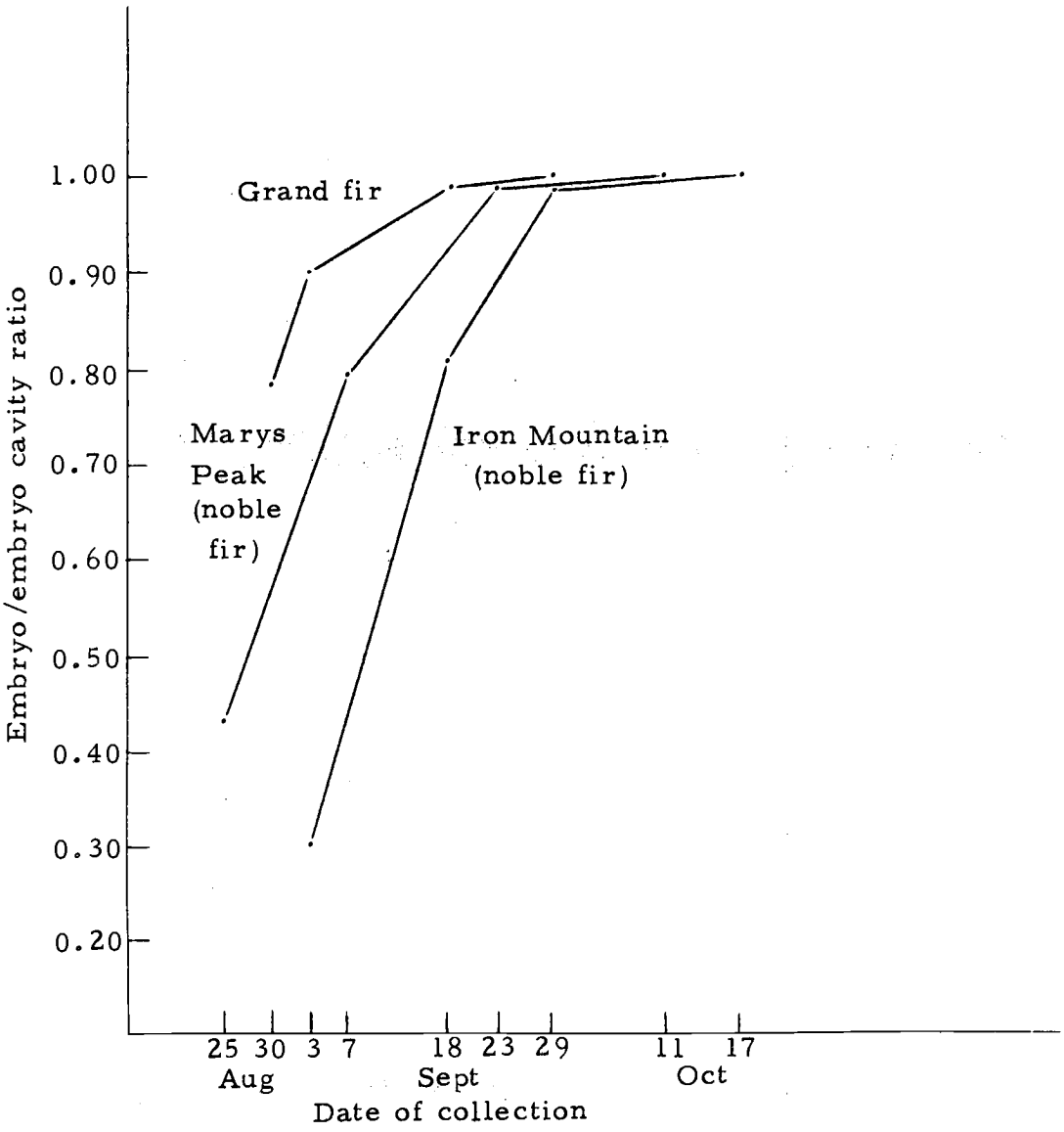


Figure 5. Embryo/embryo cavity ratio: grand fir and noble fir.

most cases. In the case of cones that were subject to outdoor storage mature embryo development occurred by the time the cones began to open, approximately four weeks after the last collection date (Table 2). The ratio of embryo length to embryo cavity length was highly correlated with total germination of sound seed ($r = 0.94$).

Seed Germination

The changes in germination occurring with date of harvest and type of storage are illustrated in Figures 6 and 6A, 7 and 7A. As is indicated on the ordinate in each figure, the germinability is expressed as a percentage of the total number of full, sound seed in each sample, i.e. only actual germination is shown.

Actual germination is the number of seeds germinating as a fraction or percentage of the number of viable seeds, germinated seeds plus tetrazolium stained seeds, sown. This contrasts with apparent germination, which is the number germinating as a fraction of the total number of seeds sown.

Seed germination for the noble fir seed source in the Cascade Mountains is not shown due to its erratic and discontinuous nature. Upon microscopic examination of the seed during embryo measurements it was found that the seed was heavily infested with seed chalcids (Megastigmus sp.). This particular insect deposits its eggs by inserting its ovipositor through the cone scale and into the young

Table 2. Embryo/embryo cavity ratios.

	Date of collection	Embryo/embryo cavity ratio (treatments)		
		Fresh	Dry	Moist[a]
Marys Peak (noble fir)	Aug 12	-	0.99	0.99
	25	0.43	0.99	0.98
	Sept 7	0.79	0.99	0.99
	23	0.99	1.00	1.00
	Oct 11	1.00	1.00	-
Iron Mountain (noble fir)	Sept 3	0.30	1.00	0.98
	18	0.81	0.99	0.99
	29	0.99	0.99	1.00
	Oct 17	1.00	1.00	-
Grand fir	Aug 30	0.78	0.98	0.98
	Sept 3	0.90	0.98	0.99
	18	0.99	1.00	1.00
	29	1.00	1.00	-

[a]Measurements for the artificial-ripening treatments were made approximately four weeks after the last collection dates.

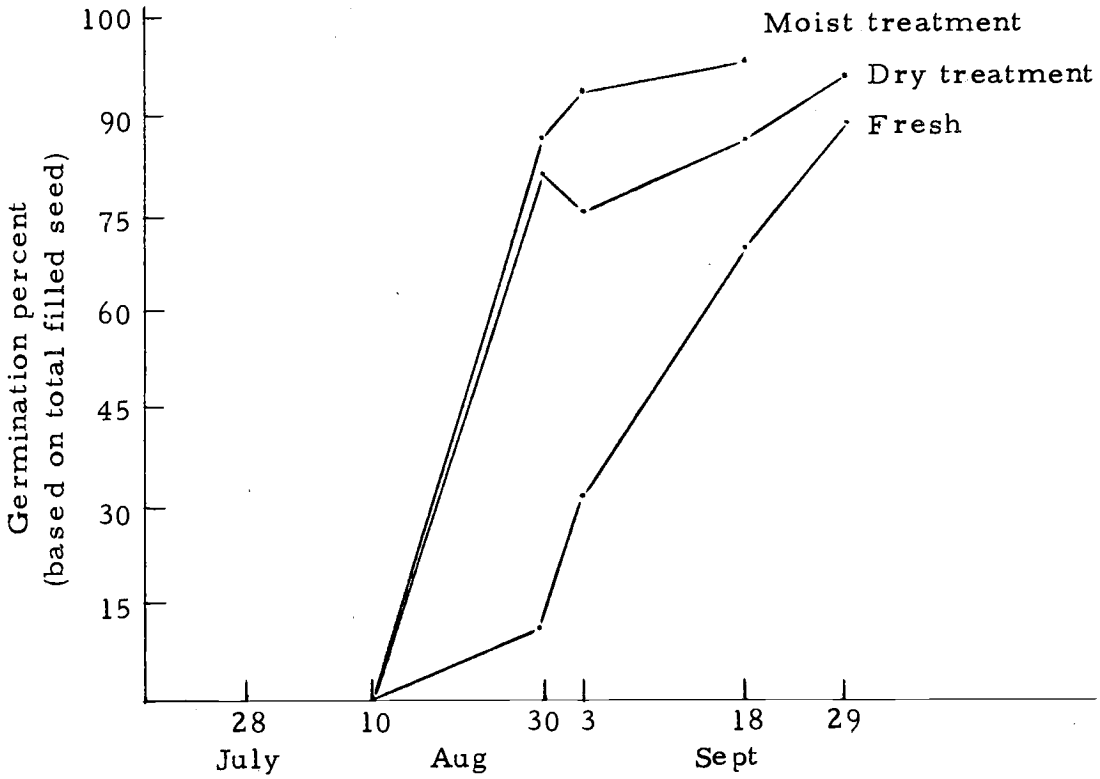


Figure 6. Germination of stratified grand fir seed.

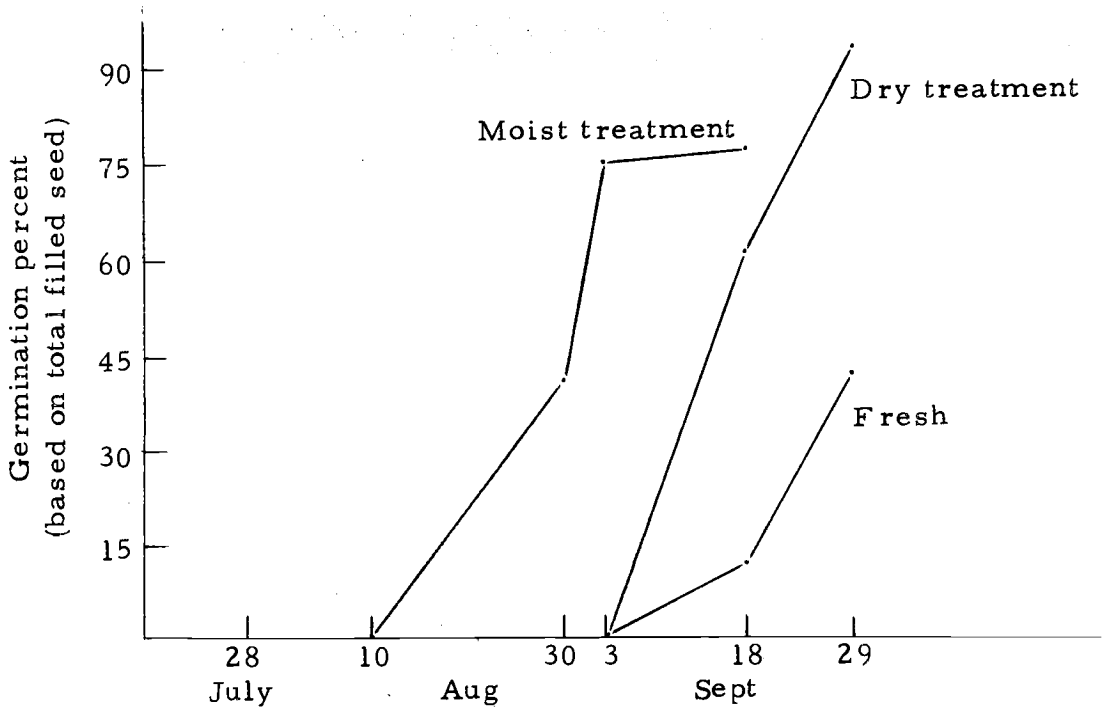


Figure 6A. Germination of non-stratified seed of grand fir.

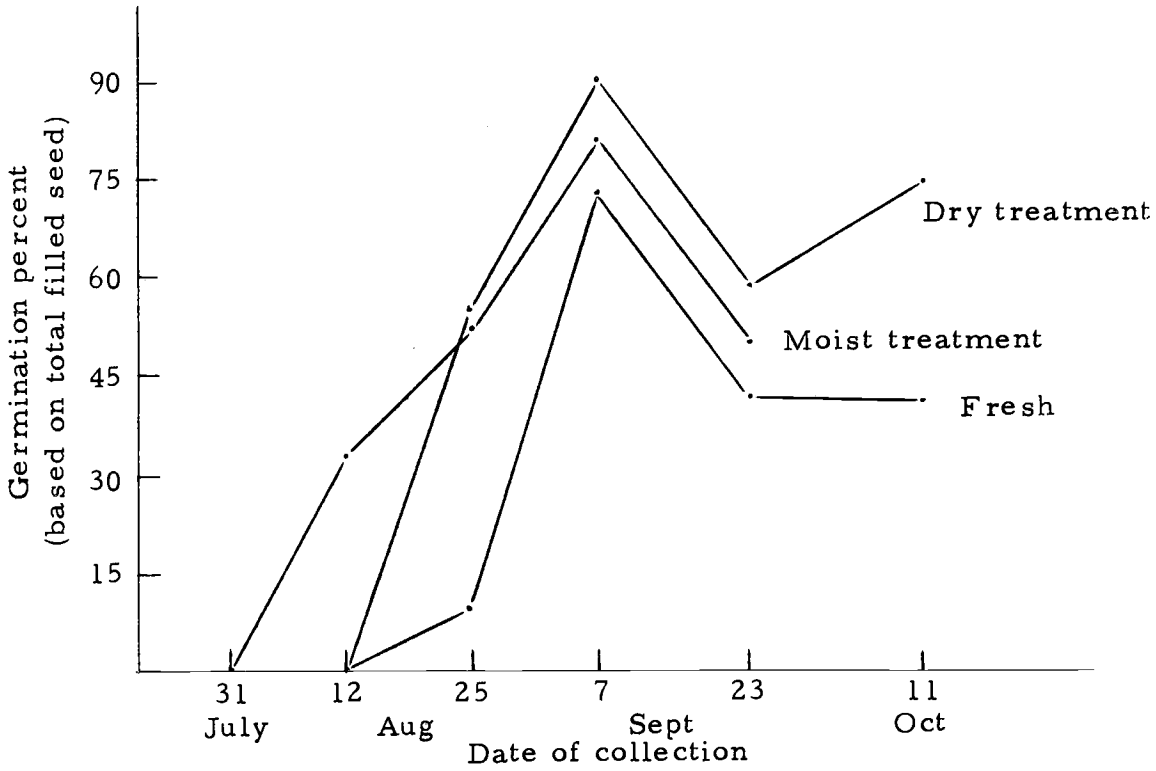


Figure 7. Germination of stratified noble fir seed.

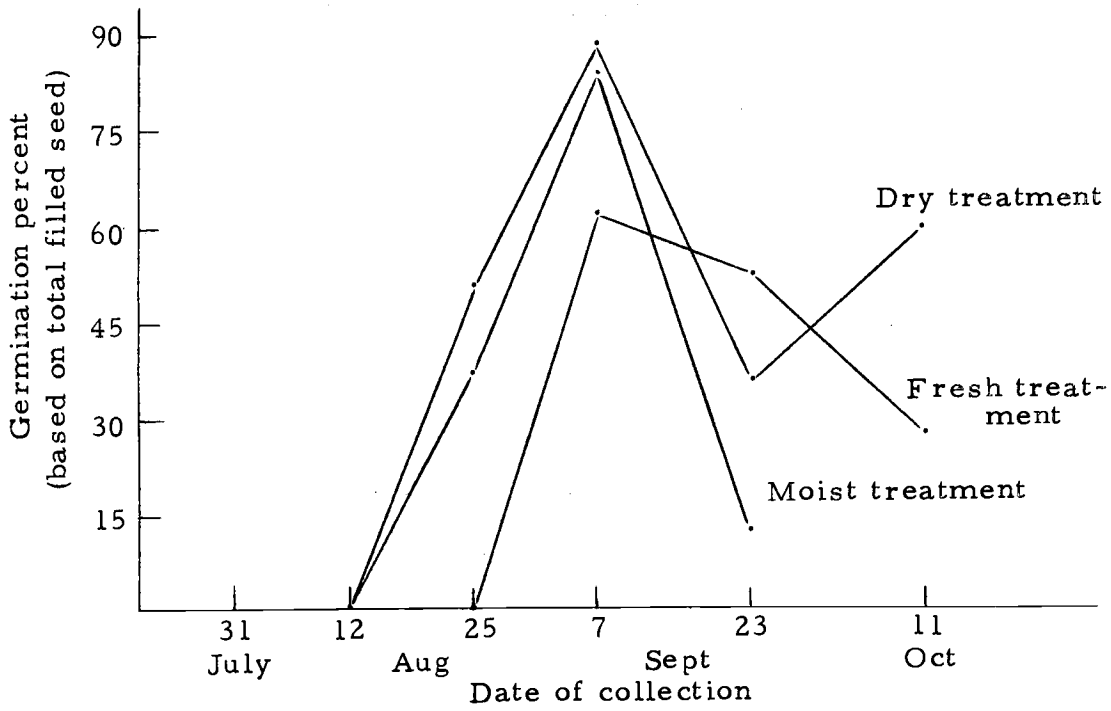


Figure 7A. Germination of non-stratified noble fir seed.

seeds in the spring. Eggs hatch in late June and early July and single largae develop in each seed and devour the embryo and female gematophyte. In an effort to control the infestation, picked cones were soaked in a one percent solution of Meta-Systox-R[3] for one hour prior to outdoor storage. However, by the time the cones in the latter part of the study were treated substantial damage had already been done. Consequently, seed germination was negligible in several instances for this seed source. Seed chalcids were also found, to a lesser degree, in the noble fir seed collected from the Oregon Coast Range.

The effects of seed stratification in almost all cases were consistent; more rapid and a higher number of seeds germinated than unstratified samples (Table 3). In addition, the germination capacity, the number of germinated seeds plus the viable seed, was almost always higher in the stratified samples.

Germination of both unstratified and stratified grand fir seed tended to increase with date of harvest as shown in Figures 6 and 6A. This species showed a peak germination of freshly collected seed on the last collection date. Following storage of the cones, germination was even more markedly increased. The effects of stratification, as indicated by the increase in germination over non-stratified seed,

---

[3]Meta-Systox-R is a Chemagro product.

Table 3. Actual germination, germination capacity and germination rate of noble fir and grand fir seed.

Collection date and storage treatment	Actual germination (based on total filled seed)		Germination capacity		Germination rate	
	S[a]	NS[a]	S	NS	S	NS
			Grand fir			
August 30	12.5	-	15.7	12.5	0.83	-
D[b]	81.6	28.6	83.4	38.4	14.8	1.2
M[b]	86.8	40.0	89.9	50.0	15.7	4.7
September 3	31.8	-	50.0	16.7	3.8	-
D	71.9	-	87.8	76.4	36.2	-
M	94.3	75.0	96.2	92.9	59.3	18.9
September 18	69.9	11.5	83.6	49.1	20.2	1.4
D	86.1	61.4	91.7	76.2	75.0	16.6
M	94.9	76.6	97.5	90.6	58.4	24.6
September 29	89.2	41.9	92.8	87.4	51.2	9.4
D	96.0	93.0	97.9	93.0	66.3	23.2
			Noble fir			
August 12	-	-	-	-	-	-
D	-	-	-	-	-	-
M	33.3	-	33.3	34.4	1.0	-
August 25	10.0	-	10.0	12.3	1.0	-
D	55.0	51.1	65.7	60.0	11.5	6.4
M	53.8	37.0	70.4	56.1	9.5	7.0
September 7	73.3	62.5	73.3	62.5	13.4	3.1
D	90.5	89.1	95.2	92.0	23.5	14.7
M	81.8	84.0	81.8	91.3	10.2	5.6
September 23	41.7	52.9	61.9	55.0	5.7	3.6
D	59.3	36.8	66.7	58.4	7.2	3.2
M	50.0	12.5	50.0	52.5	8.1	3.1
October 11	40.0	28.6	57.1	57.2	3.7	2.0
D	75.0	60.0	87.5	60.0	3.7	2.3

[a] S signifies stratified seed, while NS signifies non-stratified seed.

[b] D signifies seed stored under dry conditions, while M signifies seed stored under moist conditions.

however, appear to diminish with storage. From these data, it appears that outdoor storage of the cones, particularly under moist conditions, followed by seed stratification is the most beneficial treatment.

In the case of noble fir, germination of the seed increased with time until early September and then decreased until seed was fully mature. The reduction in germination of seed on the last collection dates cannot be explained from available data. These cones were handled in precisely the same manner as those of earlier collections. Franklin (1965) encountered a similar decrease in average germination of noble fir seed as cone development progressed. Regardless of this fact, it may still be seen that storage of the cones also increased seed germination. It would appear that dry outdoor storage of cones, followed by seed stratification, is the most beneficial treatment for obtaining maximum germination.

Seed extracted from grand fir cones on September 3 and from noble fir cones on August 25 had an average germination of 32 percent and 10 percent respectively. With the same lot of cones, if the seed was allowed to remain in the cone stored in the shade outdoors, the germination increased from 32 percent to 72 percent and 91 percent under dry and moist conditions respectively. Similarly, the germination of noble fir seed increased from 10 percent to 54 percent during outdoor storage. Thus, it was apparent that this seed was immature

when picked (Table 2), but by allowing the seed to remain in the cone, maturation continued.

In the present study, stratification also had a pronounced effect on germination rate (Table 3). The larger values found for the stratified seed reflect more rapid germination. There was also an increase in germination rate for both stratified and non-stratified seed from cones which were subject to outdoor storage. Both outdoor storage of the cones and seed stratification enhanced the germination rate over seed which had been extracted from the cone immediately after each collection.

Seedling Vigor

The vigor and quality of grand fir and noble fir seedlings were correlated with maturity of the cones; maximum weight and a balanced root to shoot ratio were realized during the last collection prior to seedfall (Table 4). During the collection period, seedling vigor, as measured by oven dry weight of seedlings, increased as maturity approached. Also, seed which had been subject to outdoor storage in the cone showed greater vigor than that seed which was extracted immediately after collection. Noble fir seed collected September 23 and allowed to after-ripen (outdoor storage) produced more vigorous seedlings than those from seed collected in October. Likewise, grand fir seeds collected on September 18 and stored outdoors appeared to

Table 4. Seedling vigor of noble fir and grand fir seed.

Collection date and storage treatment	Average oven dry weight of seedlings (mg)		Average root/shoot ratio		% Survival of germinated seed sown	
	S[a]	NS[a]	S	NS	S	NS
			Grand fir			
September 3	20.5	-	1.3	-	75.0	-
D[b]	22.9	-	1.5	-	83.9	-
M	21.5	18.8	1.5	1.4	83.7	47.1
September 18	28.1	22.2	1.5	1.5	87.5	66.7
D	32.2	29.3	1.7	1.6	95.9	85.2
M	29.4	27.3	1.8	1.9	88.5	75.3
September 29	25.8	22.2	1.8	1.4	85.7	65.6
D	32.5	29.5	1.7	1.6	94.3	79.1
			Noble fir			
August 25	-	-	-	-	-	-
D	31.8	28.5	2.2	2.3	76.2	50.0
M	33.0	30.1	1.7	2.0	77.7	61.2
September 7	25.3	21.1	1.4	2.0	81.2	44.4
D	21.2	25.1	1.4	1.4	93.3	85.7
M	27.5	26.4	1.3	1.4	100.0	79.8
September 23	31.6	24.0	1.9	1.7	82.6	51.7
D	39.0	26.6	2.2	1.6	85.7	31.2
M	38.3	28.0	1.9	1.6	75.0	65.7
October 11	38.0	36.4	1.6	1.6	100.0	50.0
D	28.0	26.8	0.9	1.5	100.0	50.0

[a] S signifies stratified seed and NS signifies non-stratified seed.

[b] D signifies seed stored under dry conditions and M signifies seed stored under moist conditions.

be more vigorous than seed extracted from the cone immediately after the last collection.

Table 4 also illustrates that the percent survival of sown germinants increased for both species as date of seedfall approached. In addition, the percent survival of seedlings from seed which had been subject to outdoor storage in the cone was greater than seed extracted immediately after collection. Apparently, the outdoor storage of cones was beneficial in increasing the seed vigor.

There was a tendency for seedlings from stratified seed to be more vigorous than those from non-stratified seed. Both average oven dry weight of seedlings and percent survival of sown germinants were higher for the stratified seed of both species (Table 4). There was also a general increase in seedling weight for those seedlings from stratified and non-stratified seed which had been subject to outdoor storage conditions. The increase in seedling weight may reflect normal seed weight trends which have been observed in coniferous tree seed studies (Ching and Ching, 1962; Pfister, 1967).

Seed Vigor

Estimates of time required to reach given percentiles of germination were computed from the germination data by using the Weibull function (Dell and Bonner, 1976). A longer time period was required to reach a given germination percentile for non-stratified

seed of both species, indicating lower seed vigor in seeds not subject to a prechill treatment (Table 5). In the case of grand fir, those seeds subjected to outdoor storage in the cone attained given germination percentiles sooner than seed extracted from the cone immediately after collection, indicating increasing seed vigor for artificially-ripened seed. Results for noble fir seed were similar but faster germination, as indicated by shorter periods required to reach selected germination percentiles, was not observed until the latter part of the germination period.

Biochemical Analyses of Seed Material

From each collection of cones, both prior to (fresh) and following storage (dry and moist artificial ripening), one gram samples of embryo and female gametophyte tissue without seedcoat were subjected to chemical analyses. Figures 8 to 13 show the changes of biochemical constituents during the course of this experiment.[4] Many of the early collections of cones that were subject to artificial ripening produced no mature seed, thus there is are no biochemical analyses data for these seeds.

From the early embryo stage (late July) until the time of cotyledonary development (late August) there is an increase in the free

---

[4]Exact quantities of chemical constituents are found in Appendix Table 5.

Table 5. Days required to reach given percentiles of germination for noble fir and grand fir.

Collection date and storage treatment	Days to germination percentiles 0.25	0.50	0.75	0.90
		Grand fir		
September 3	21	25	28	30
D[a]	10	15	22	28
M[a]	16 (13)[b]	9 (17)	12 (22)	15 (25)
September 18	10 (22)	14 (24)	19 (26)	23 (28)
D	7 (19)	9 (23)	12 (26)	15 (28)
M	8 (16)	11 (20)	14 (24)	17 (27)
September 29	9 (19)	12 (22)	16 (25)	20 (28)
D	6 (18)	10 (21)	14 (24)	19 (26)
		Noble fir		
August 25	10	13	15	18
D	9 (9)	11 (11)	12 (14)	14 (15)
M	4 (10)	5 (11)	6 (13)	8 (14)
September 7	6 (13)	9 (18)	12 (22)	17 (27)
D	8 (10)	11 (14)	13 (17)	15 (20)
M	8 (9)	11 (12)	14 (16)	17 (17)
September 23	5 (12)	8 (15)	12 (18)	16 (21)
D	5 (14)	7 (16)	10 (18)	12 (19)
M	4	6	8	9
October 11	5 (13)	6 (15)	6 (17)	7 (19)
D	5 (12)	5 (14)	6 (16)	7 (18)

[a]D signifies seed stored under dry conditions and M signifies seed stored under moist conditions.

[b]Numbers in parentheses signify seed subject to no stratification.

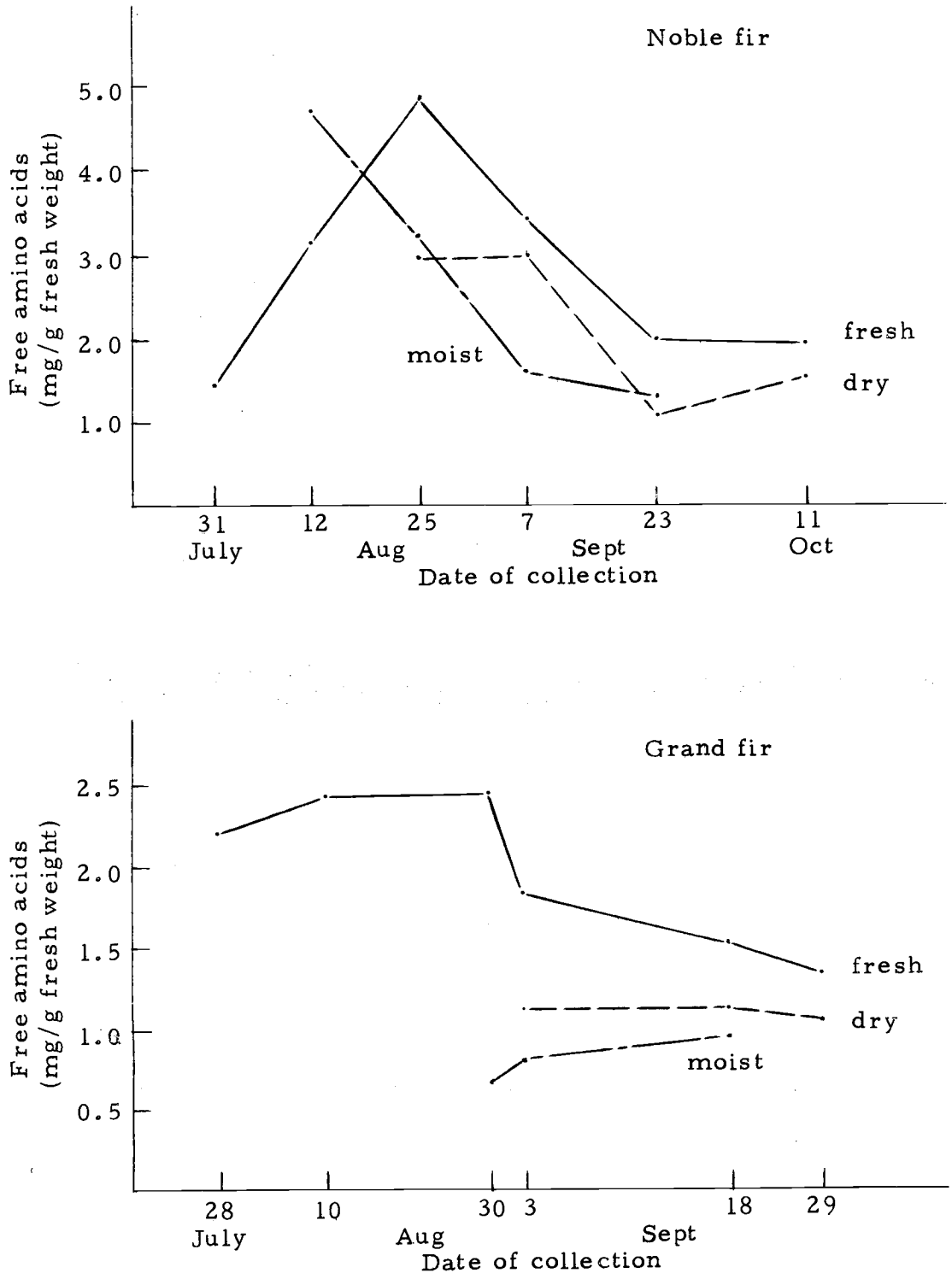


Figure 8. Free amino acids in seed tissue.

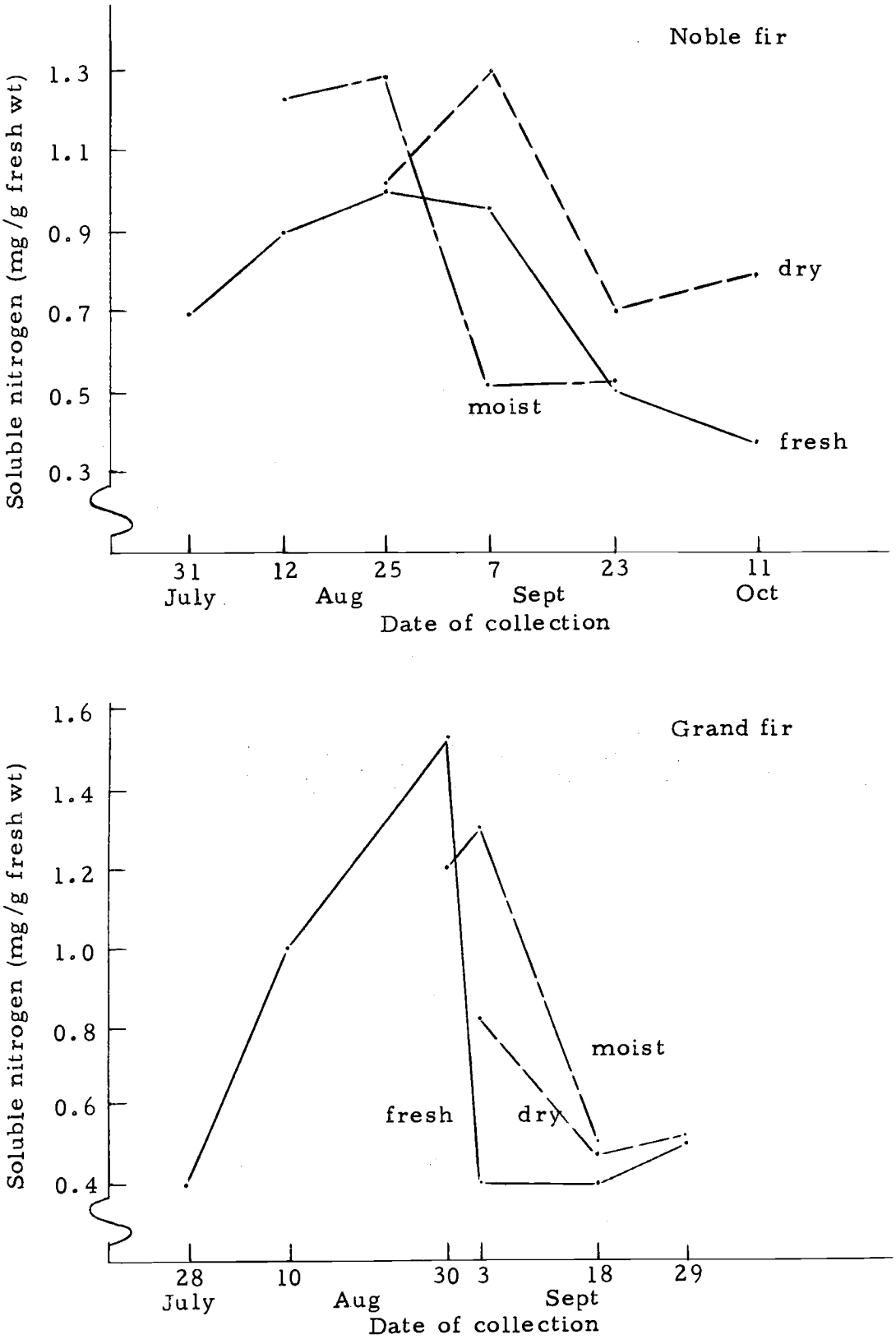


Figure 9. Soluble nitrogen in seed tissue.

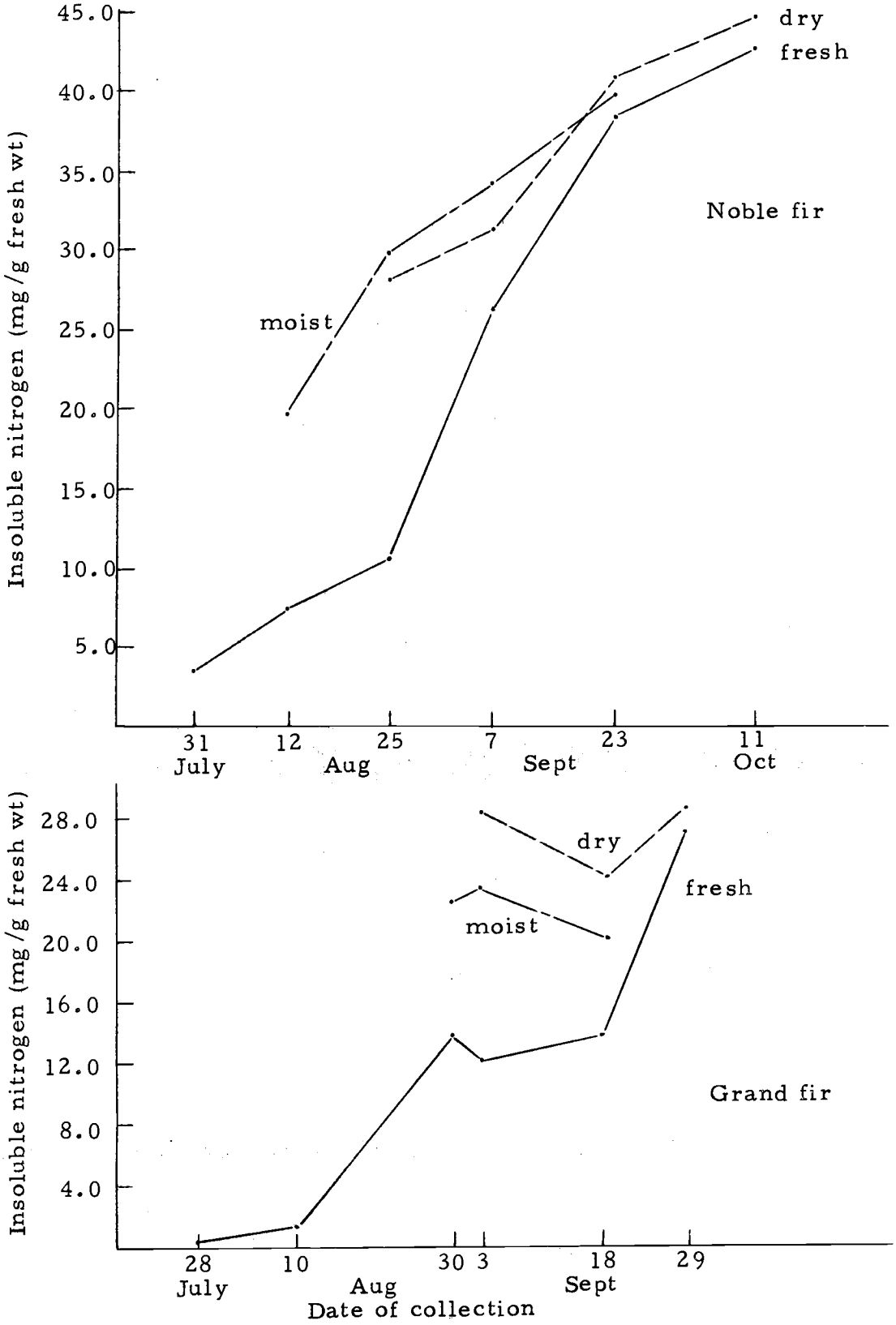
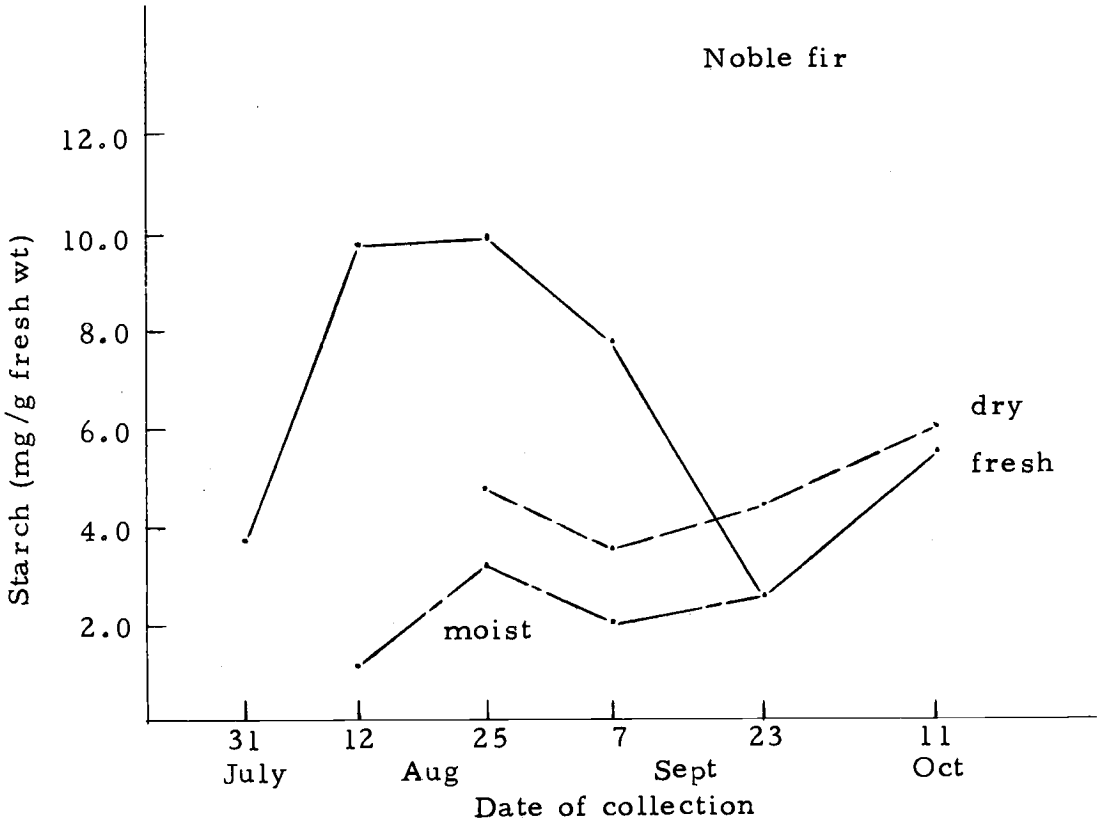


Figure 10. Insoluble nitrogen in seed tissue.



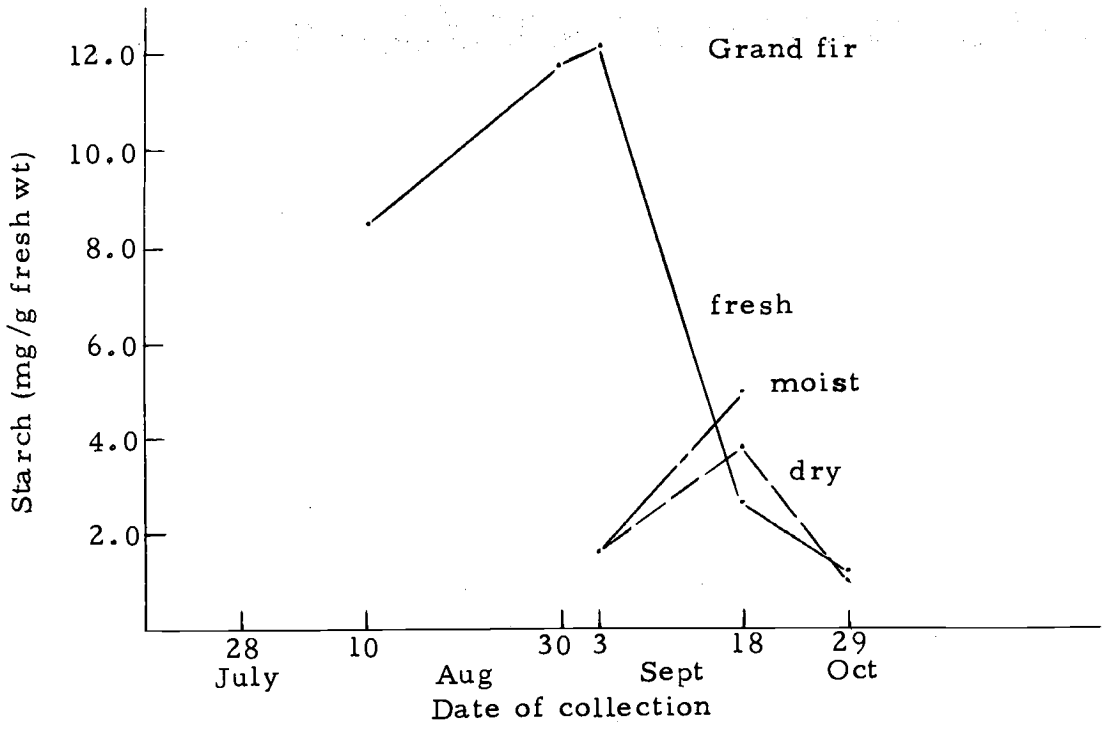
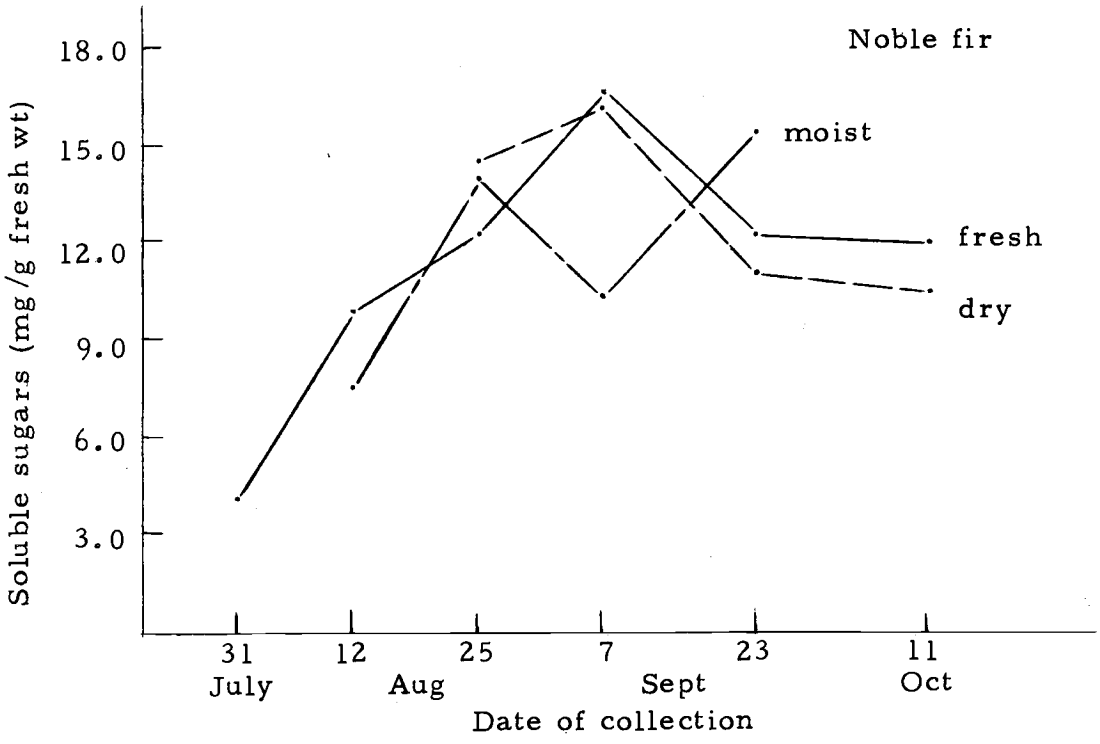


Figure 11. Starch in seed tissue.



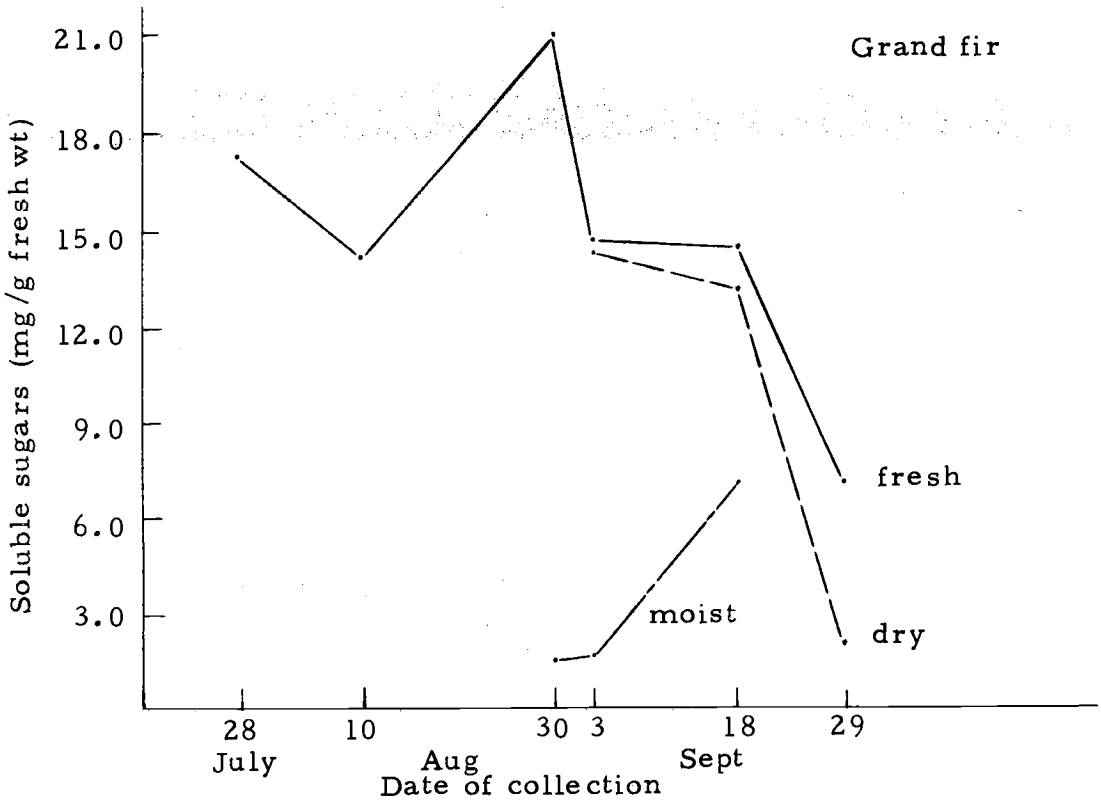


Figure 12. Soluble sugars in seed tissue.

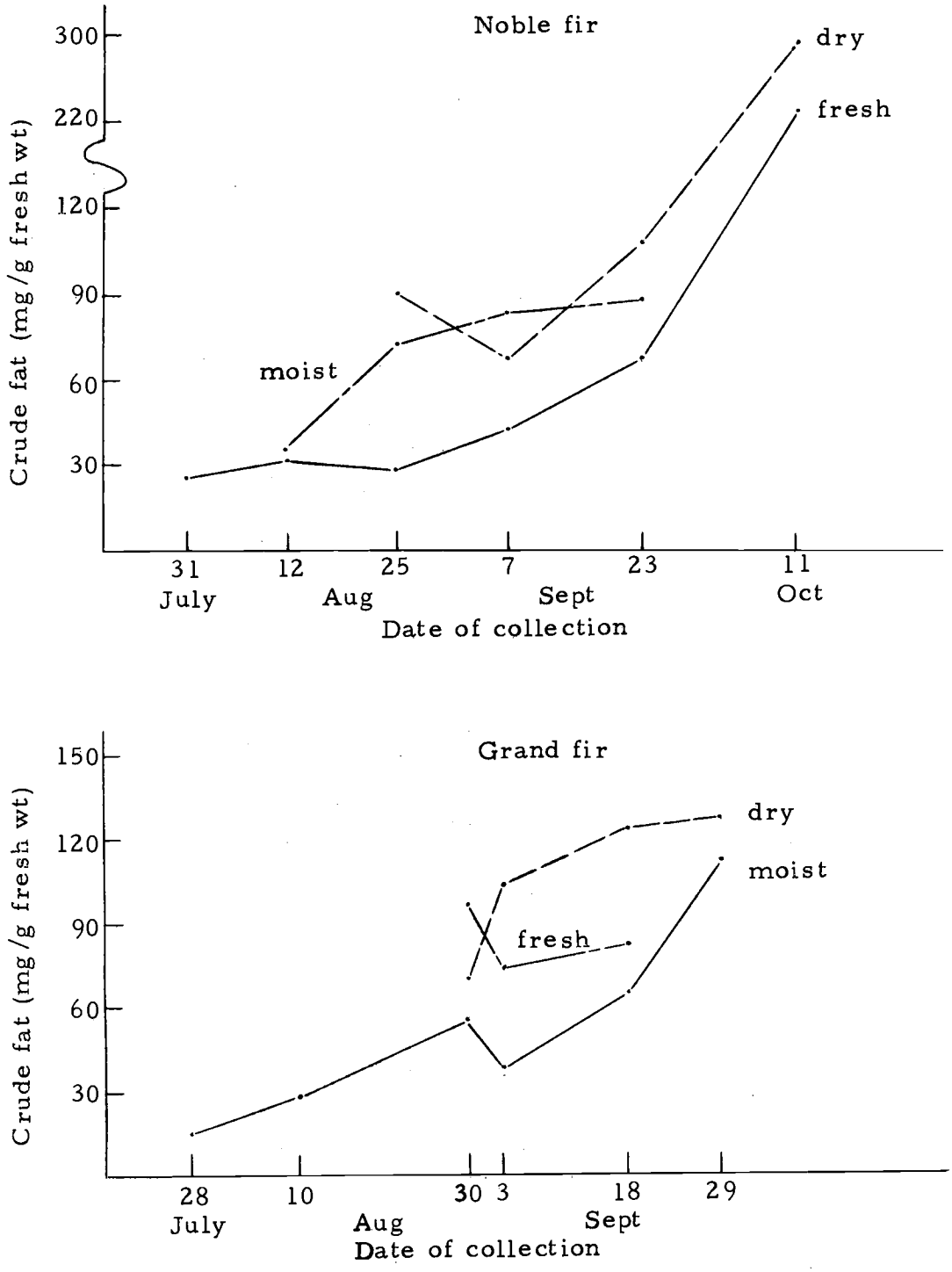


Figure 13. Crude fat in seed tissue.

amino acid content in the seed tissue of fresh material (Figure 8), of both species. After that the quantity of free amino acids gradually falls to a minimum value on the last collection dates. Grand fir seed from cones subjected to a period of outdoor storage (artificially ripened) showed a free amino acid content less than those seeds that were analyzed in the fresh condition. In the case of noble fir, the minimum content of free amino acids on the last collection date (1.9 mg/g) was not realized in seed subject to artificial ripening until the early September collection of the moist ripening treatment. Due to the fact that all artificially ripened seed was analyzed at the same time in November, four weeks after the last collection, a somewhat constant low level of amino acids would be expected, as was seen in the case of artificially ripened grand fir seed. Consequently, the high content of amino acids in artificially ripened noble fir seed during August and early September indicates incomplete transformation. In general, the disappearance of free amino acids with maturity indicates the synthesis of proteinaceous reserves at the expense of amino acids.

Similar rise and fall patterns of soluble nitrogen during embryo development were shown in both species (Figure 9). A continuous increase of protein nitrogen of seed material was observed (Figure 10). This is quite understandable as the more mobile forms of nitrogen are probably being synthesized into various proteins for structure,

reserve material and enzymes. Consequently, as the seed matures the insoluble nitrogen content increases.

During artificial ripening the insoluble and soluble nitrogen increased beyond those levels found in fresh seed material. For noble fir, seeds which were harvested in late September and allowed to artificially ripen in the cone reached nitrogen levels which were greater than those found in fresh seed examined after the last collection. Seeds collected prior to this time, however, did not attain as high a level of protein nitrogen in their tissue and their seedling vigor, as measured by oven dry weight, was also not as great (Table 4). This suggests that immature seeds reach a stage when they no longer must depend on the tree for nutrition and organic substances for further development. Grand fir seed collected in early September and subjected to artificial ripening in the cone also attained levels of nitrogen greater than those found in fresh seed at the time of seed dispersal. Apparently then, during this period of artificial ripening there is a further increase in the proteinaceous compounds within the seed.

Katsuta (1961) reported that the rapid transfer of nitrogenous substances into Pinus thunbergii seeds was utilized in embryo development, and emphasized that transformation of soluble nitrogen compounds to protein nitrogen was important in increasing the germinative capacity of seeds as they matured.

Starch levels tended to decrease as maturation progressed (Figure 11). In the case of noble fir, however, there was a slight increase in starch levels at the final collection date. Artificial ripening of cones, of both species, had various effects on starch levels. For noble fir, all seed that was artificially ripened, except during the last collection, exhibited starch levels less than those found in fresh seed just before seed dispersal. Grand fir seed collected in early September and artificially ripened showed starch levels less than fresh seed harvested at that time, but greater than fresh seed collected at the final collection date. In contrast to this, seed collected from mid-September on attained starch levels greater than fresh seed collected at that time, as well as during the final collection. The somewhat erratic nature of starch content could possibly be seen more clearly if the moisture content of the seed tissue and the dry seed weight had been measured. Starch may function as an important substrate for the carbon backbone of structure and reserves of developing seed.

When the embryo started to develop (early September) the soluble sugar content decreased and shortly thereafter crude fat rapidly increased and continued to increase with maturity (Figures 12 and 13). During artificial ripening soluble sugar levels decreased more than those of fresh seed. However, minimum levels of sugars that were measured on the last collection dates for both species

were not realized in artificially ripened seed until the latter part of September.

During artificial ripening of the cones crude fat content also increased more than the increases observed in fresh seed. Grand fir seed collected in mid-September and allowed to artificially ripen in the cone under dry conditions attained crude fat levels that were higher than those found in fresh seed on the last collection date. These seeds were also more vigorous than fresh seed collected on the last collection date (Table 4). In the case of noble fir cones subjected to artificial ripening, levels of crude fat equal to those of fresh seed at the last collection were not realized until a week before the last collection. However, seeds collected in mid-September and artificially ripened showed more vigor than those collected on the last collection date and treated as fresh (Table 4). This is unexplainable since crude fat is the major reserve of coniferous tree seeds and it would seem logical that the more reserves a seed contained, the more vigorous it would be. As can be seen from these data, even after the cones have been picked their seeds show a general increase in crude fat levels, reflecting a conversion of cone scale reserves to seed storage and structural materials.

Biochemical Analyses of Cone Scale Material

Much of the food material which was accumulated in the latter stages of developing seeds came from the senescing cone scales. Ching and Ching (1962) showed this to be the case for Douglas-fir seeds. Katsuta and Satoo (1964) also emphasized that cone scales of Pinus thunbergii seemed to be reservoirs for nitrogen transferred from leaves and branches to developing seeds. Cone scales, being modified branches, have vascular connections to the cone axis where photosynthates and minerals for growth and development can be readily obtained.

During the time period of this study there was a decrease in soluble sugars and free amino acids in the cone scales of both species, as seeds matured (Figures 14 to 17).[5] These contents were even further reduced in artificially ripened material. In almost all cases for both species, levels of soluble sugars and amino acids decreased below those levels found in the cone scale during the final collections, suggesting that mobile forms of organic material were being completely translocated into the developing seed in the artificial ripening process. It is known that a decrease in carbohydrates of maturing strobili is associated with mobilization of reserves by seeds (Ching and Ching, 1962; Krugman, 1966).

---

[5] Exact quantities of chemical constituents are found in Appendix Table 6.

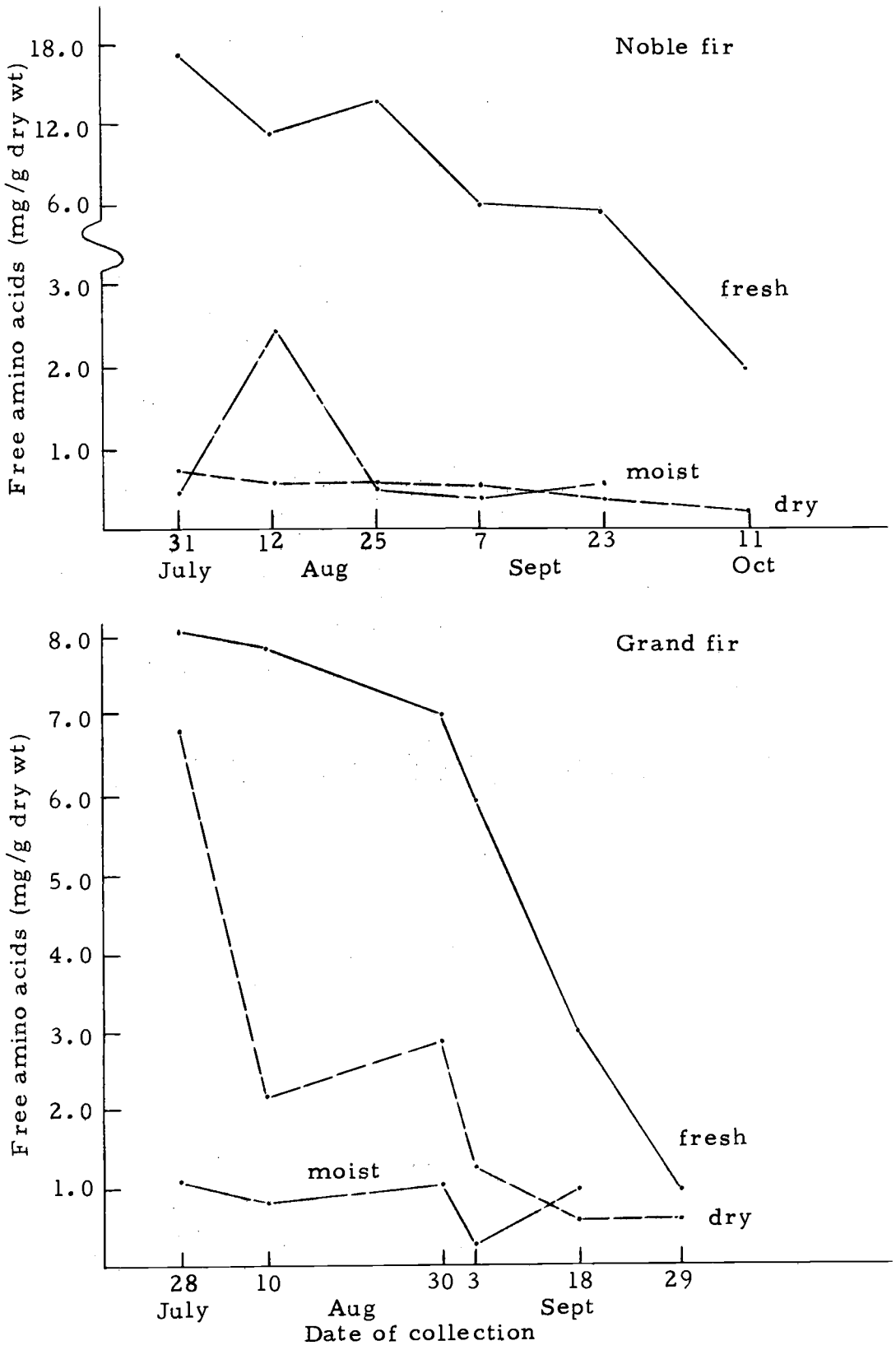


Figure 14. Free amino acids in cone scale material (dry weight).

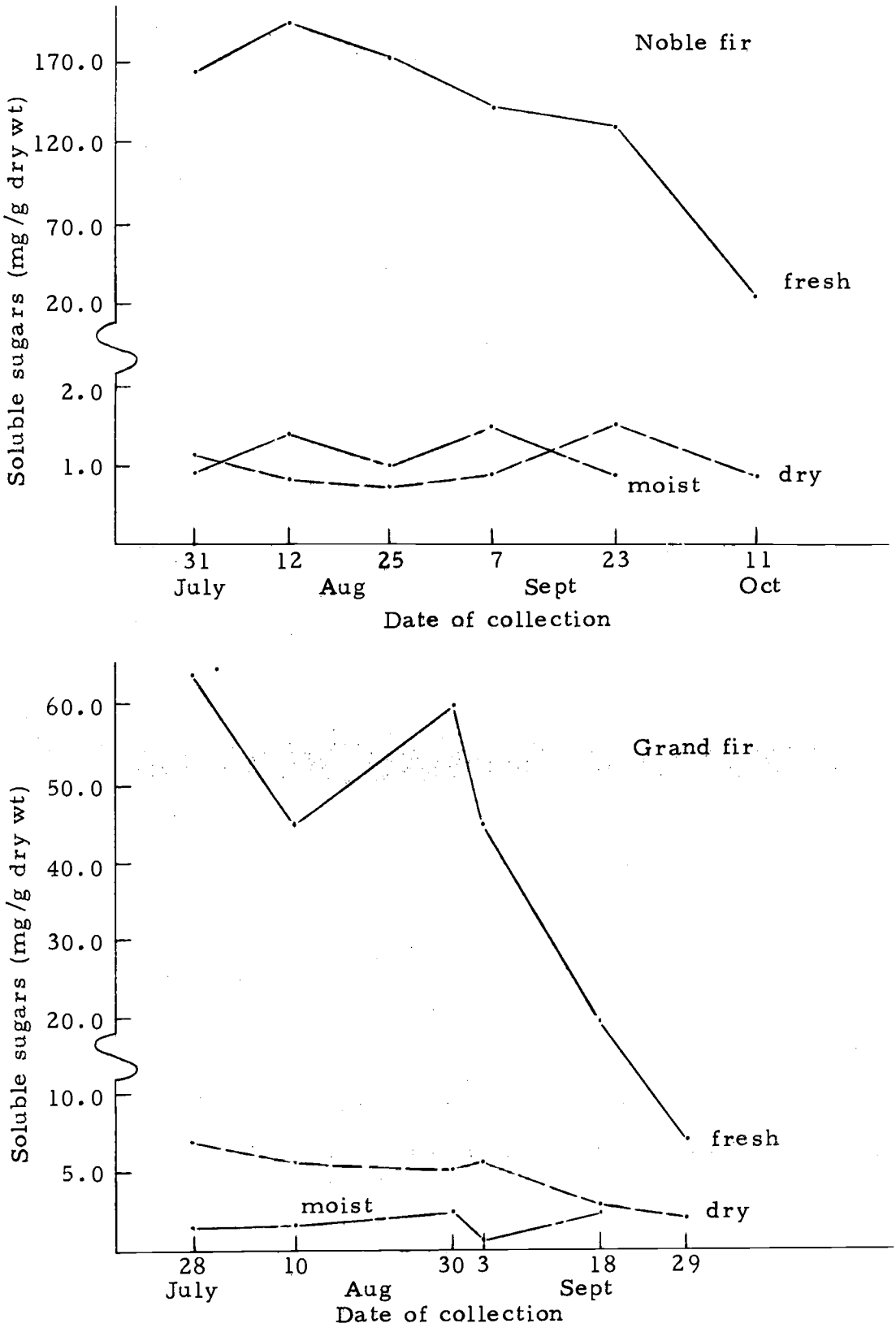


Figure 15. Soluble sugars in cone scale material (dry weight).

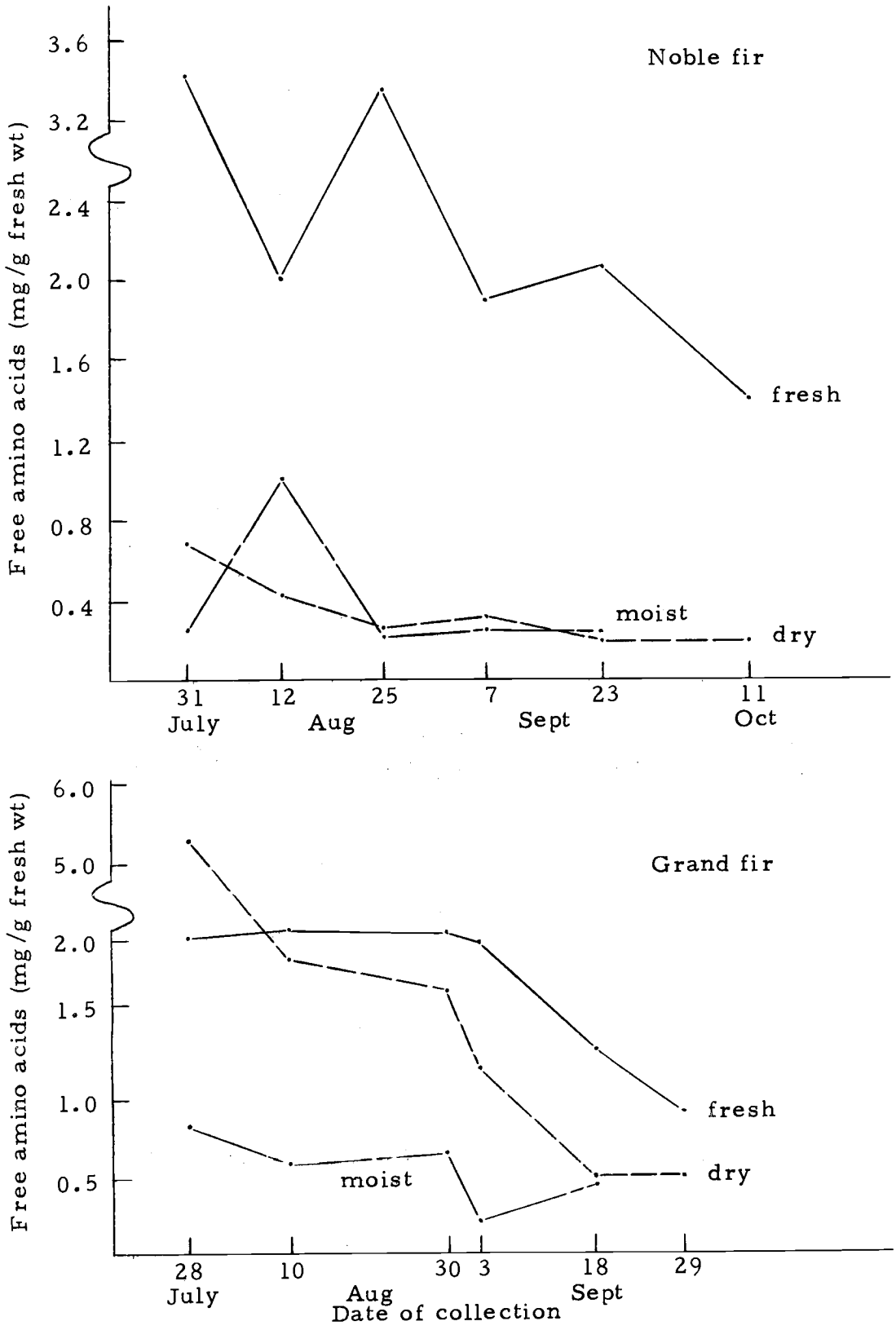


Figure 16. Free amino acids in cone scale material (fresh weight).

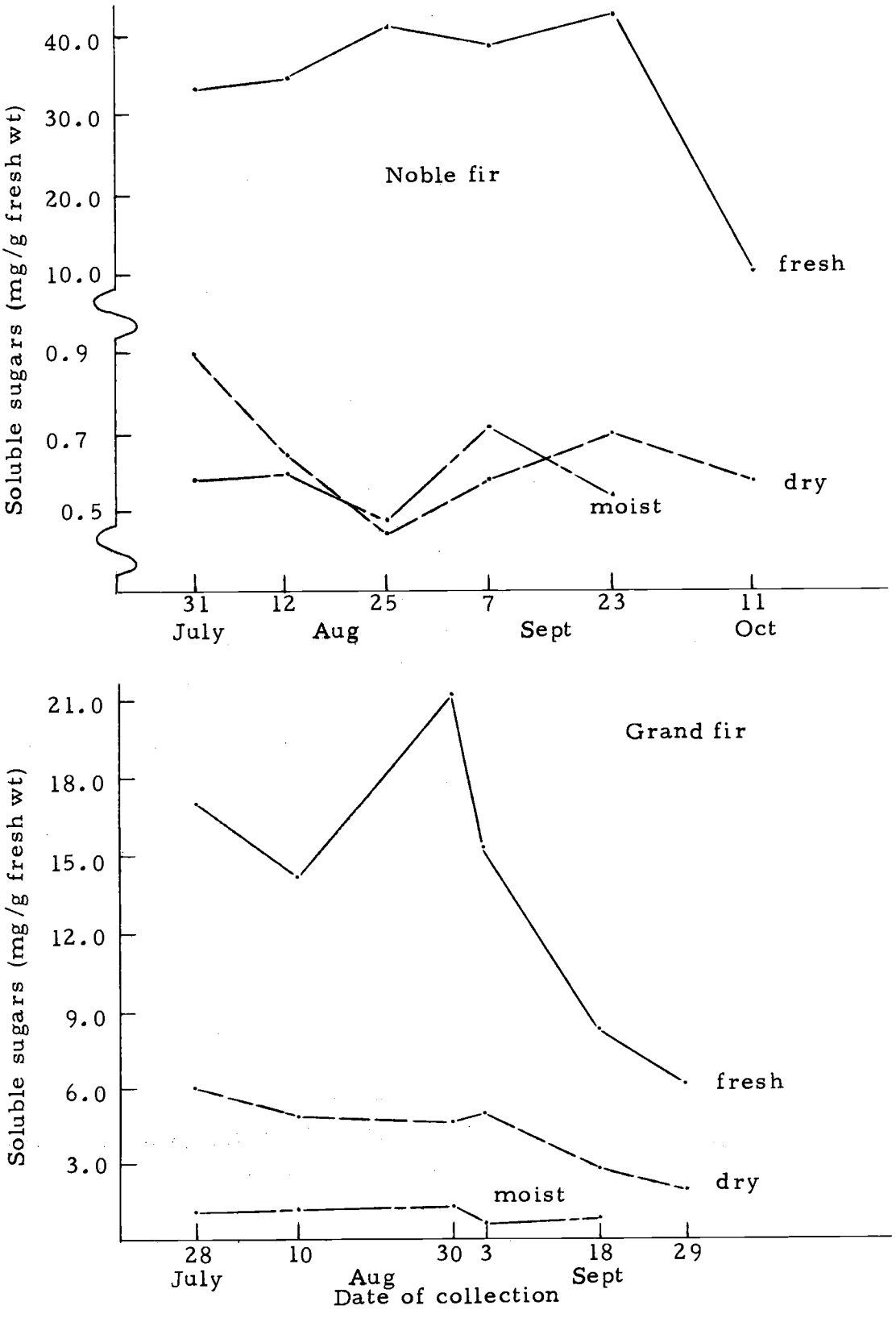


Figure 17. Soluble sugars in cone scale material (fresh weight).

In the case of noble fir, there was also a decrease in soluble nitrogen levels of cone scales as seed fall approached (Figures 18 and 19). Cones collected throughout the period and artificially ripened had soluble nitrogen levels that were lower than those levels found in cone scales at seed fall, based on dry weight. For grand fir similar trends were shown only under moist outdoor storage conditions. Under dry artificial ripening levels of soluble nitrogen did not reach minimum levels until mid-September.

Protein nitrogen content for both species showed a continuous decreasing trend during the cone collection period (Figures 20 and 21). It is obvious that in young cone scales less woody tissue resulted in higher nitrogen contents. In artificially ripened cones higher protein nitrogen content was noted, and the longer the ripening period, the higher the nitrogen content. This is difficult to explain from the available data.

Starch levels in cone scale material of both species decreased as seed matured (Figures 22 and 23). The minute levels of starch found in this material decreased to almost nothing by the last collection date. In artificially ripened cones the starch content was lower than that of fresh cone scale material. However, the starch content of artificially ripened material did not always attain the level of starch measured in cone scales collected on the final date.

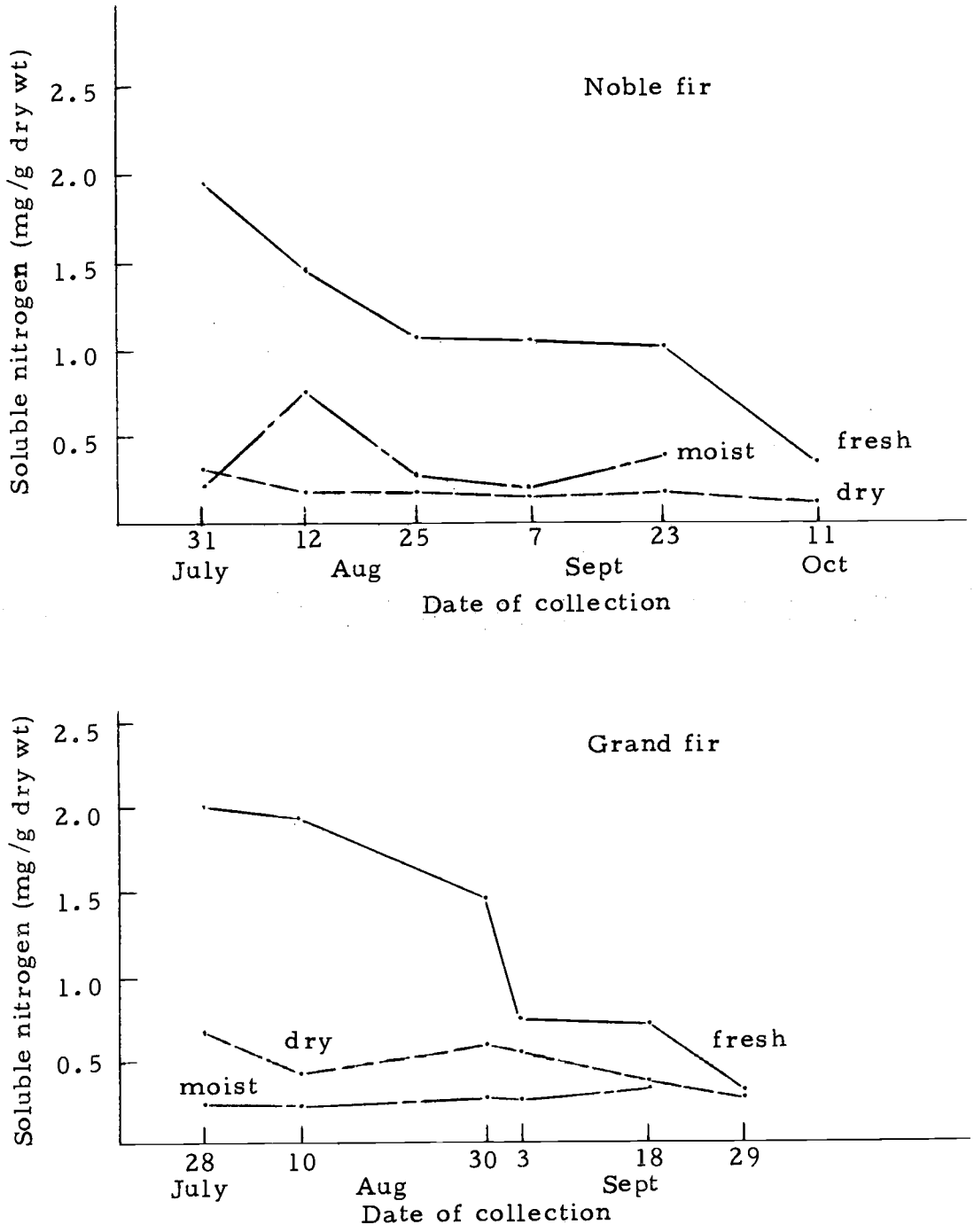


Figure 18. Soluble nitrogen in cone scale material (dry weight).

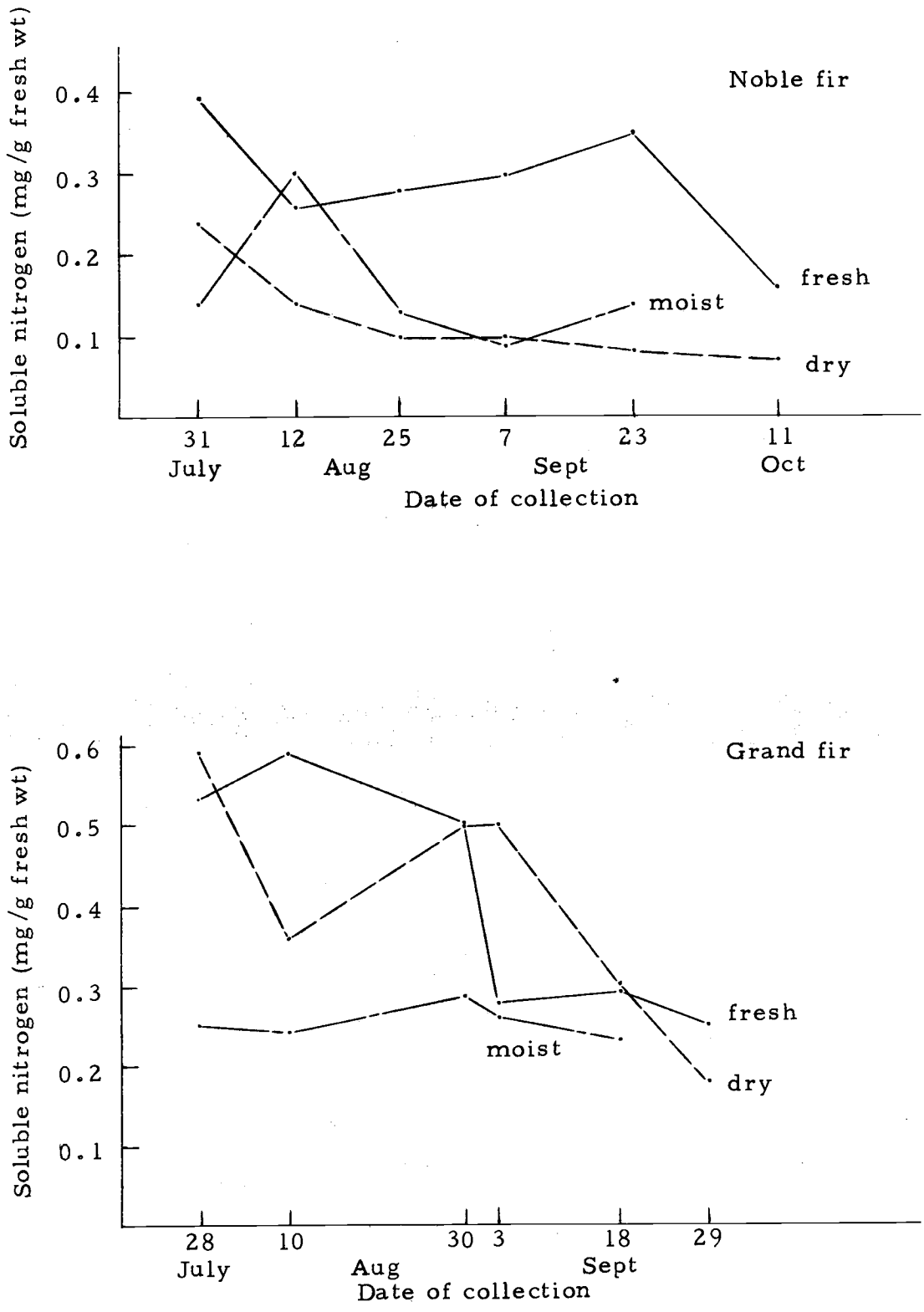


Figure 19. Soluble nitrogen in cone scale material (fresh weight).

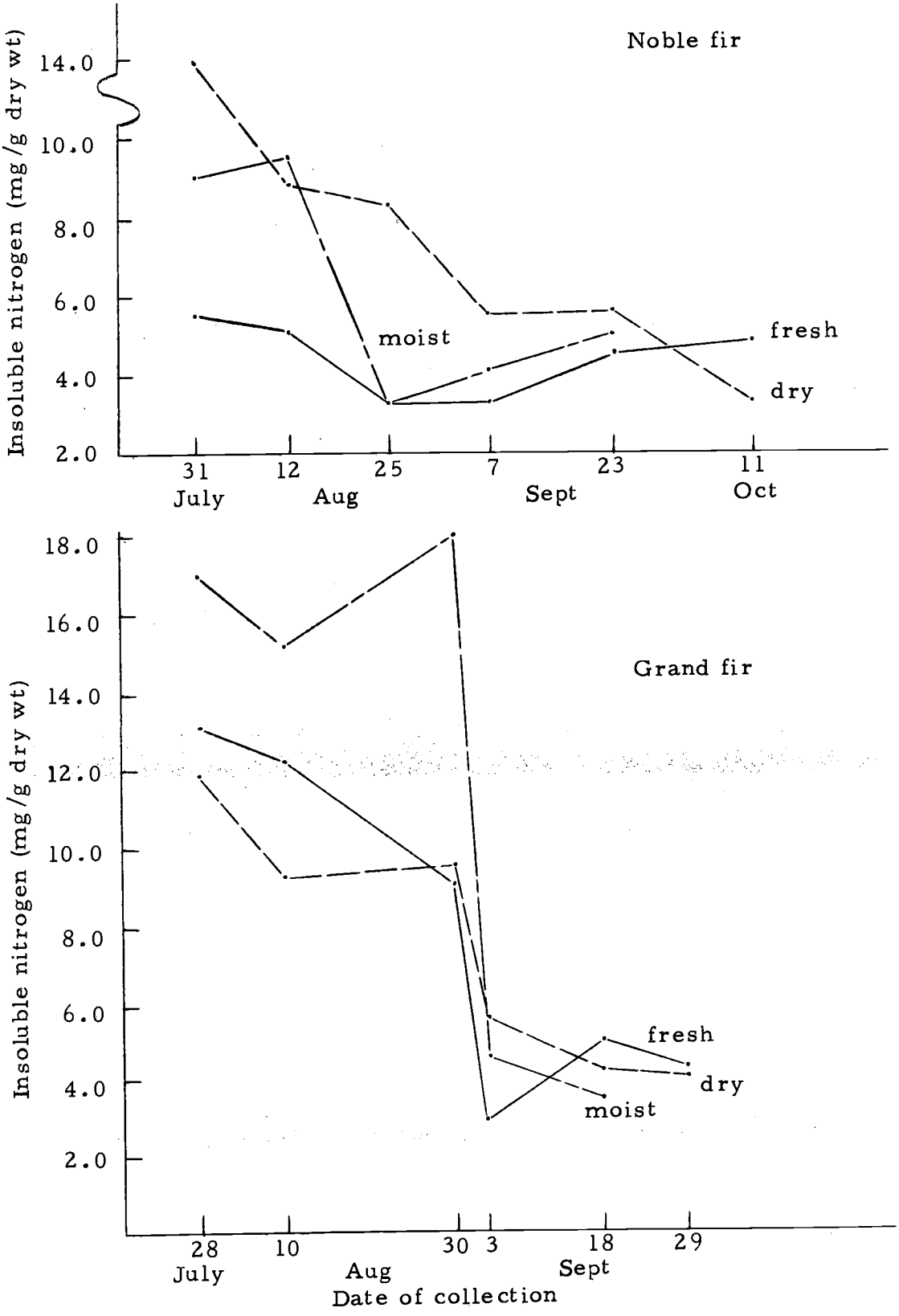
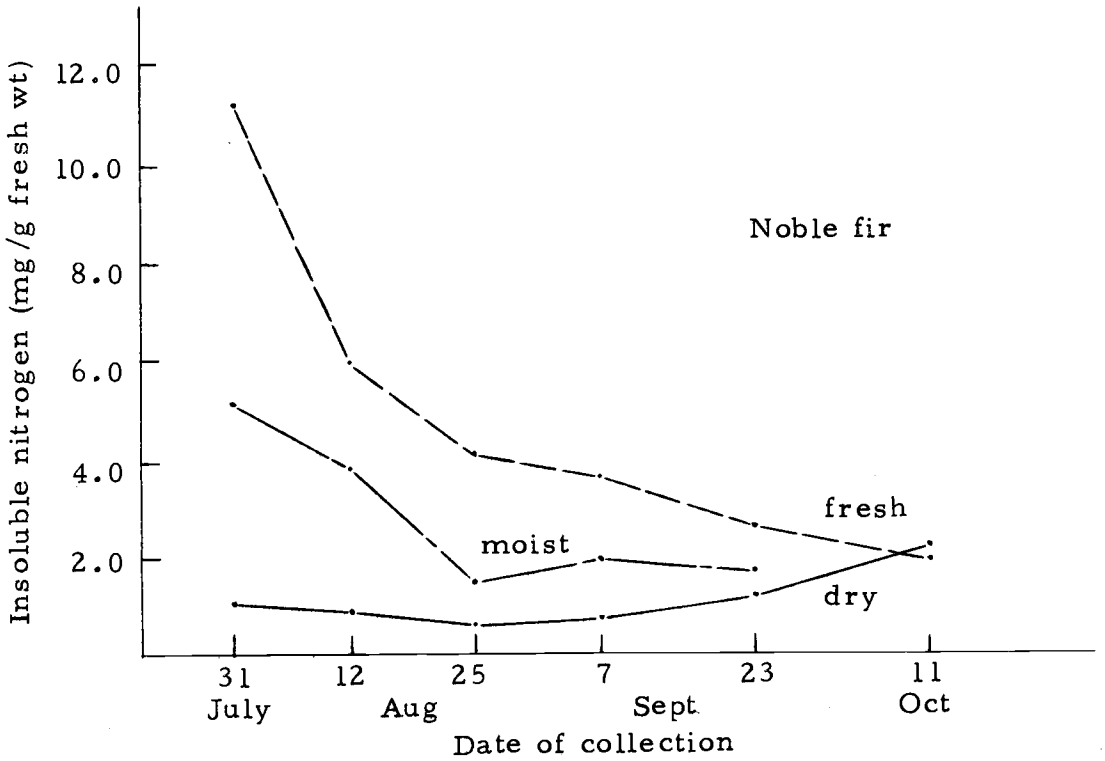


Figure 20. Insoluble nitrogen in cone scale material (dry weight).



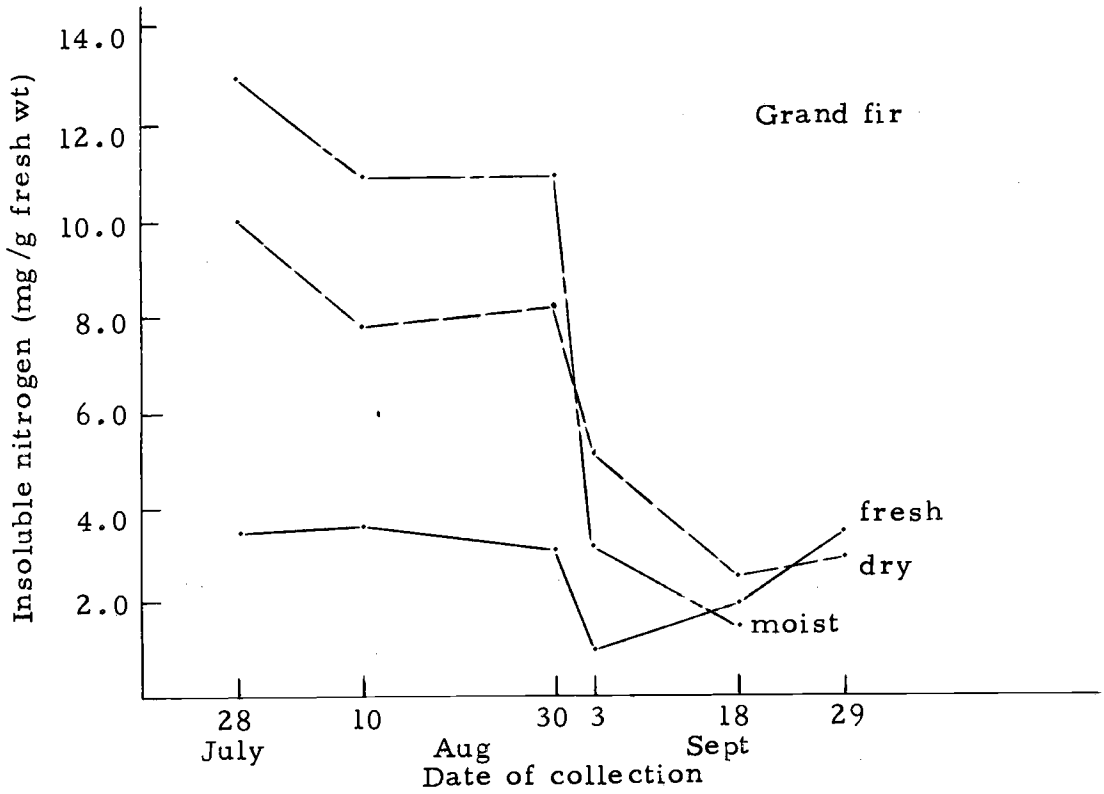


Figure 21. Insoluble nitrogen in cone scale material (fresh weight).

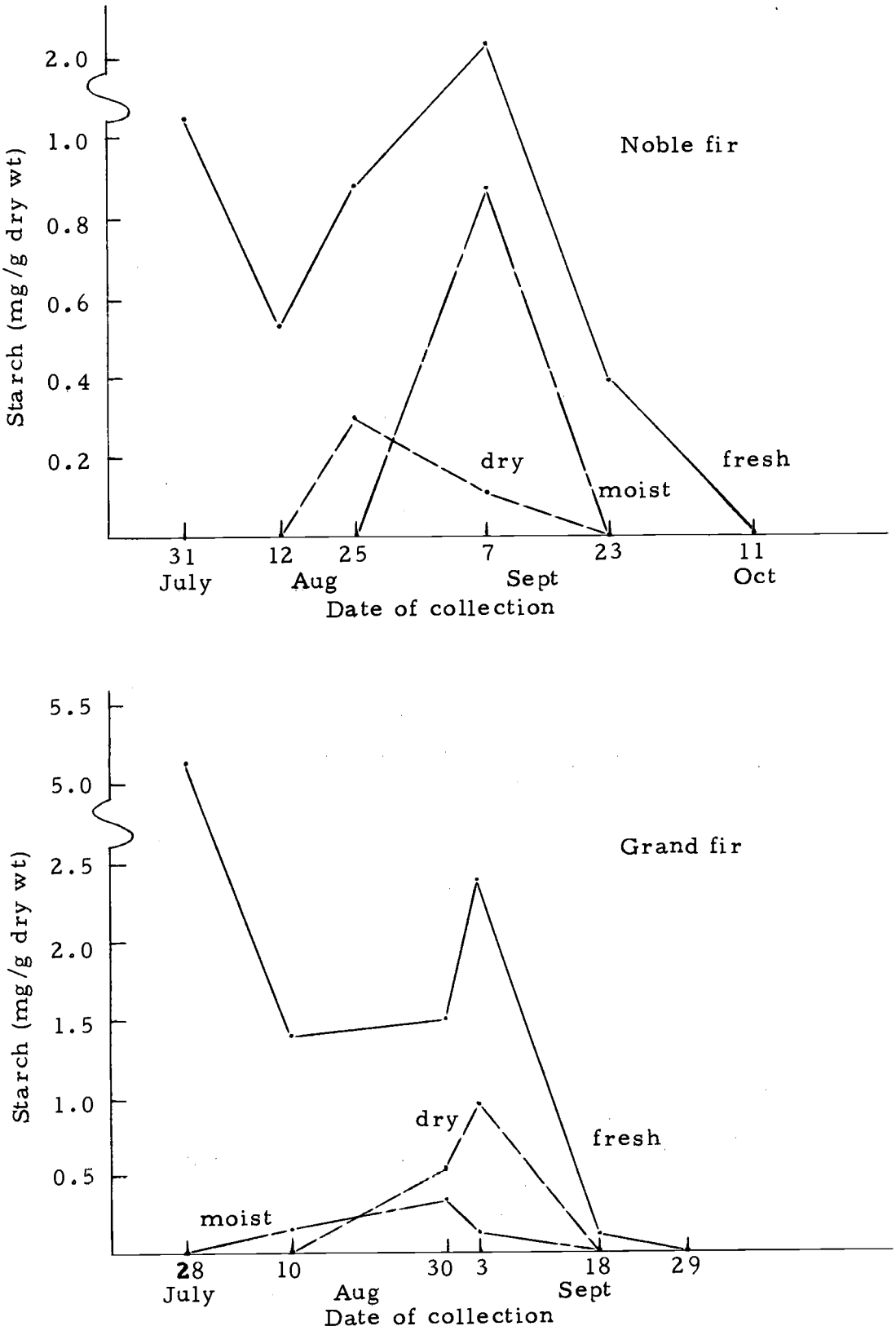


Figure 22. Starch in cone scale material (dry weight).

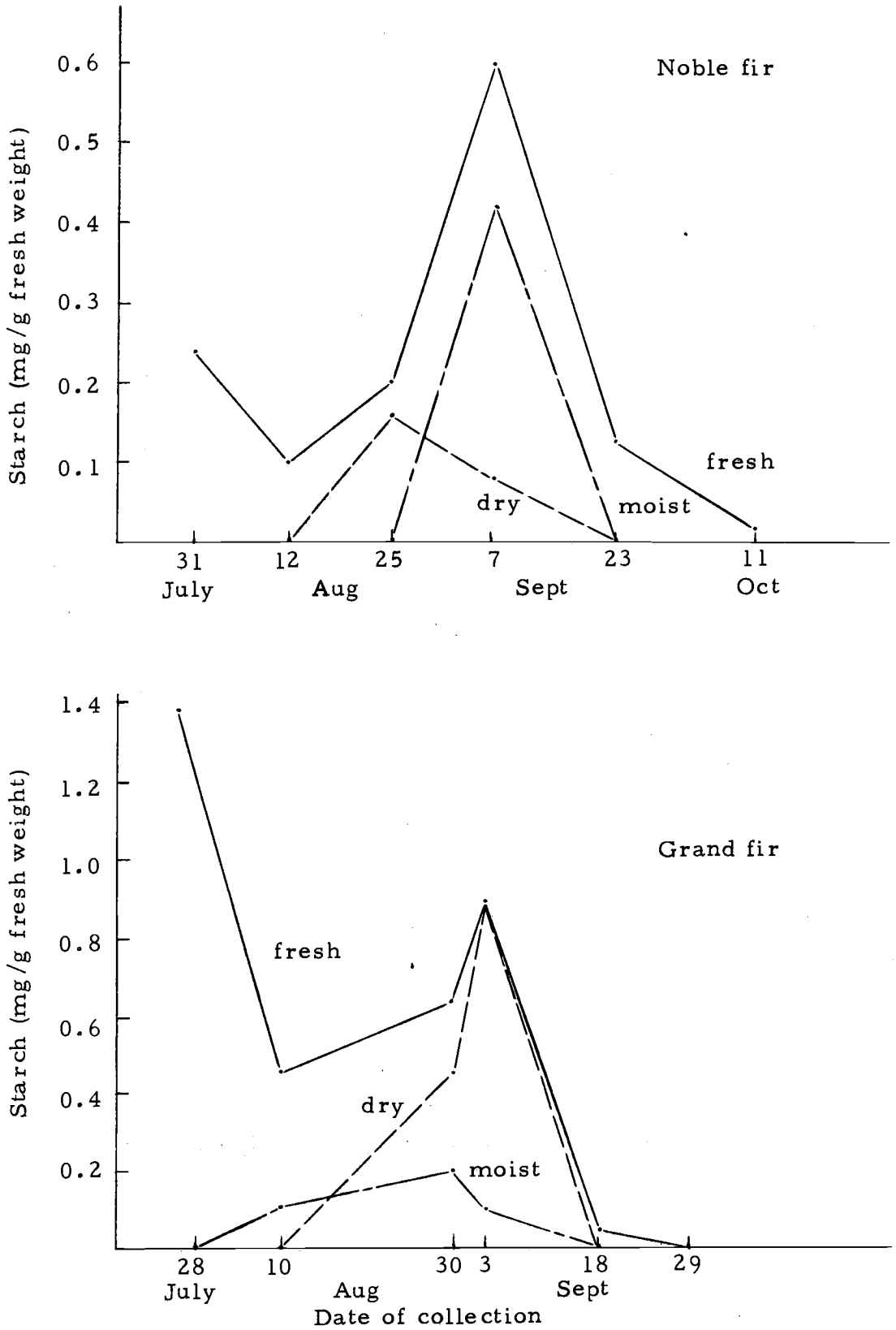


Figure 23. Starch on cone scale material (fresh weight).

The decrease in reserve materials of cones probably was associated with mobilization of these materials by maturing seed. Seeds undoubtedly accumulated organic material from the cone scale throughout the season and during the artificial ripening period, as evidenced by the increase in embryo development and the increase in seedling vigor. This translocation of material out of the cone scales must occur prior to cone shattering.

DISCUSSION

Seed Germination and Maturity Indices

It is generally known that cones of Abies species have scales and bracts which are deciduous at maturity. It is essential to harvest prior to this disintegration, but the timing of the collection is of considerable importance since it has been observed that germinability tends to increase up to cone shattering. This may vary with species; Oliver (1974) for white fir, noted that germination continued to increase right up to the time of seed dispersal, whereas in noble fir (Franklin, 1965) germination tended to level off as the time of dissemination approached. This also appeared to take place in Douglas-fir (Rediske, 1961).

For grand fir the increase in maturity, as measured by germination, approaches linearity without any leveling off prior to seed dispersal. Due to the erratic nature of noble fir seed germination no definitive seed germination pattern was observed.

It would appear, then, that in terms of achieving maximum germination from freshly harvested seeds, mid-September would be the optimum harvest time for grand fir cones under similar environmental conditions found in the study area. Assuming noble fir seed germination levels off prior to seed dispersal as mentioned previously,

late September would be the best time to initiate harvest operations for noble fir cones.

The germinability of freshly harvested seed, as well as seed subjected to artificial after-ripening, was increasingly enhanced by stratification. This may be interpreted as demonstrating an increase in seed dormancy with advancing harvest date. Thus, as the seed ripens stratification improved seed germinability by breaking dormancy, which had steadily deepened. For most of the seedlots stratification promoted faster and more complete germination, a feature of coniferous seed verified in numerous places elsewhere in the literature.

Due to the various changes in biochemical constituents in the seed and the improved germination and seed vigor, there is little doubt that changes did occur in the fir cones maintained under the storage conditions described in the Materials and Methods portion of this thesis.

Outdoor storage proved to be beneficial in extending the ripening process in most all of the collections. Krugman (1966) reported similar artificial ripening of sugar pine seeds stored in the cone at $10^{o}C$. Although not all immature seeds could be ripened in this way it clearly showed that seeds were no longer entirely dependent upon the mother tree for nutrition.

In the case of noble fir, greatest improvements in germinability occurred in seeds of cones which had been subject to outdoor storage under dry conditions. Observations made on late-August cones showed that it is possible that these collections would have yielded viable seed if suitably stored. All cones collected during the remainder of the study appeared to continue the ripening processes and to yield higher quality seed if artificially ripened. For commercial collectors it would probably be best to begin cone collections in the latter part of September when there would be a higher percentage of developed seed in the cones. This seed has been shown to be more vigorous than seed from earlier collections and seedling survival is higher.

For grand fir, the greatest seed germinability occurred when the cones were subjected to moist outdoor storage. However, this particular artificial ripening treatment may not be practical for commercial operators. First, this particular treatment would require more time, space and money in order to set up than does the present system of dry storage in a cool, shaded location. And secondly, and perhaps most important, is the fact that total yield of sound seed may be lower than that produced by dry storage. The constant moisture around the basal portion of the cone completely destroys the seeds found there. Consequently, less cleaned, sound seed per cone resulted than under the dry storage treatment. Seeds stored under

dry conditions proved to be more vigorous than those subject to moist outdoor storage.

Germinability of seed from grand fir cones collected in mid-September and stored under dry conditions outside yielded seed with as high a germination percentage as seed collected just before cone disintegration. Seed vigor of these seeds, as measured by seedling weight and germination rate, was similar to those seeds collected at seed dispersal. Commercial cone collectors could begin collections in mid-September and if these cones were properly handled high quality seed could be obtained.

Later collections of seed produce more dormant seed and require stratification even after a period of outdoor storage. From September on the degree of dormancy became advanced, probably as a survival mechanism of the species. Outdoor storage under cool conditions did continue to increase seed quality but to a lesser degree than for earlier collections. This increase in seed quality would seem to indicate after-ripening, i.e. further changes in otherwise ripened seed.

Sufficient evidence was not available to test the hypothesis that movement of nutrients from the moist perlite into the cone did occur. And in the case of noble fir, it was found that dry outdoor storage was more beneficial than moist outdoor storage.

Of the physical characteristics of the cone and seed examined only specific gravity and the ratio of embryo length to embryo cavity length appeared to be of much immediate value in assessing seed ripeness. Specific gravity of the cone has been shown to be a fairly reliable index of seed maturity in many coniferous species (USDA, 1974). The earliest cone collections for grand fir displayed a specific gravity greater than one. As the seed matured, this value decreased rapidly and reached an observed low on the last collection date. Data in Table 3 suggest that the best time for cone collection of grand fir used in this study was during the middle of September. These seeds were extracted from freshly harvested cones having a specific gravity of approximately 0.85 (Figure 3). Similarly, noble fir cones had a specific gravity of just less than one when the experiment began. During the subsequent ten weeks this value declined rapidly. Data in Table 3 suggest that the best time for cone collection of noble fir, in this study, was during the latter part of September. Cone specific gravity at this time was 0.80 (Figure 4). In determining cone specific gravity a representative sample of cones from more than one tree should be examined.

The ratio of embryo length to embryo cavity length was another useful index of seed maturity. At the suggested time for noble fir and grand fir cone collections (late September and mid-September) the embryo occupied approximately 90 to 95% of the embryo cavity

(Figure 5). A ratio of at least 0.90 could prove to be a useful seed maturity index. This particular embryo length to embryo cavity length ratio has been recommended as an index of maturity for Douglas-fir (Ching and Ching, 1962) and white fir (Oliver, 1974). Future studies should aim toward verifying this characteristic as a seed maturity index in other conifers.

These two parameters, together with the following ones, may be useful in assessing seed maturity. When the seed wings are brown and unattached to the cone scale and the external appearance of the cone is light brown in color, the cones could be harvested and allowed to after-ripen. Cone color must be examined scrupulously because insect infested cones have a tendency to turn brown prior to seed maturation. By assessing several seed and cone characteristics the cone collector, especially an inexperienced one, could avoid collection of immature seed. It would seem quite useful then if several of these characteristics were used when interpreting seed maturity. An example of such a scheme is outlined in the Conclusion of this thesis.

Measurements of cone moisture content may not be practical as a seed maturity index. An index based upon a given percentage of moisture content would require continuous observations of changes in moisture content to determine the optimum moisture content. In view of the time and the cost of such a procedure it seems unlikely that commercial operators would use such an index.

Data presented in Figures 1 and 1A also demonstrate that cone weight is not useful as a maturity index.

The use of quantitative measurements of various biochemical constituents within the cone scale or seed seem to be of little value as an index of maturity also. The constituents of seeds are determined genetically, but the relative amounts of these constituents are dependent on environmental factors such as mineral nutrition and climate (Mayer and Poljakoff-Mayber, 1963). Durzan and Chalupa (1968) found that levels of free sugars, amino acids and soluble proteins in the embryo and female gametophyte of jack pine seed were related to climate at the seed source. Results of their study showed that different levels of chemical composition were reached in response to climate at the site of cone collection. Similar results were found in this study. Comparing the biochemical constituents found in the Coast Range noble fir seed with those of the Cascade Mountain noble fir seed (Appendix Tables 2 and 5), a significant difference can be seen in the quantitative measurements of the various constituents.

In order to adequately determine the biochemical changes which occur as maturity approaches complicated laboratory techniques must be used and such facilities for analyses are usually unavailable to seed collectors. Furthermore, this type of maturity index is not applicable to field determination. Even if facilities and time were

available, individual relationships between seed maturity and biochemical analyses would be necessary to adequately determine seed ripeness in different localities.

Rediske and Nicholson (1965) suggested a crude fat content greater than 25 percent of the dry weight indicated that noble fir seed would be viable if properly after-ripened. However, Edwards (1969) found only one-half this level of crude fat content in his study of noble fir seed. Both of these values were obtained by using whole seed samples ground in a Wiley mill. In this study only embryo and female gametophyte tissue were analyzed and the results were expressed on a fresh weight basis. During the later part of September, the suggested time for cone collection, noble fir seed material contained only seven percent crude fat. It can be seen that use of quantitative measurements of biochemical constituents may not prove very useful as a predictor of seed maturity.

Biochemical Changes Occurring in Cone and Seed

This study emphasized the major changes in composition of noble fir and grand fir cones that occurred during the last ten weeks of their development. The most marked changes occurred during the latter part of seed ripening consisted of the steady accumulation of reserves. The amounts of soluble sugars, starch, soluble nitrogen and free amino acids in seeds increased from late July until the

beginning of cotyledonary development in both species (early September for noble fir and late August for grand fir). Thereafter, their concentrations in the seed decreased as maturity approached. Apparently, carbohydrates were utilized for energy and synthesis of cellular components and reserves. During this time crude fat increased rapidly, along with protein nitrogen. Even after the cones had been picked, their seeds showed a general increase in crude fat and nitrogen levels and a decrease in amino acids and soluble sugars, thus reflecting a conversion from mobile to storage forms. Nitrogen, particularly, accumulated in significant concentrations in Abies seed, probably as protein nitrogen, as pines and other conifers characteristically accumulate large amounts of protein in their seeds (USDA, 1974). This rapid transfer of nitrogenous substances into developing seeds has been shown to be important in increasing the germinative capacity of pine seeds as they matured (Katsuta, 1961).

The results of this study suggest that immature seeds of Abies reach a stage where they no longer depend on the tree for further development. For grand fir, cones which were collected from early to mid-September until the time of natural seed fall produced viable seed if artificially ripened. Noble fir cones which were collected from mid-September on also were capable of producing viable seed if the cones were subject to a period of outdoor storage. This suggests that ripening immature grand fir and noble fir cones

artificially is possible. In fact the dry artificial ripening treatment is the general practice of cone collection and processing in the Pacific Northwest. Even though the quantity of reserves, crude fat and nitrogenous compounds, in artificially ripened seed, are not as high as those in the mature seeds of the final collections, they showed equal or better vigor than the mature seed. More complete translocation of organic materials undoubtedly occurs in seed which remains on the tree.

The decrease in organic materials of cone scales on the whole probably also was associated with mobilization of these materials by maturing seeds. There are numerous reports in the literature of movement of reserve material out of old or senescing tissue. Katsuta and Satoo (1964) found that total nitrogen content decreased markedly in maturing cones of Pinus thunbergii. Translocation of nitrogen and other reserves out of senescing leaves of deciduous trees just before leaf abscission is also well documented (Kramer and Kozlowski, 1961). In the present study, soluble sugars were found to be the major component of cone scales and their concentration decreased rapidly as seed fall approached. The decrease in carbohydrates of maturing strobili is associated with mobilization of reserves by seeds. For example, Ching and Ching (1962) noted that starch grains disappeared from scales of Pseudotsuga menziesii strobili at the time they began to increase in the developing embryo. Other constituents

within the cone scale also showed decreasing trends as seed maturity approached. An even more significant decrease in these biochemical constituents occurred after the cones had been picked and artificially ripened, suggesting translocation of substances from cone scales to ripening seeds. This is shown by the completion of embryo growth, increased germination and increased germination rate of seed collected several weeks prior to natural seed fall and subject to outdoor storage in the cone (artificial ripening). In some instances low levels of soluble constituents were not observed in artificially ripened material and translocation not completed, possibly due to the requirement of organic substances from the tree.

CONCLUSIONS

Based upon the evidence gathered in this research the following conclusions have been drawn.

1. Germination of grand fir seed increased steadily up to seed fall; noble fir seed germination was erratic and no definitive pattern was observed.
2. Cones collected from the latter part of September for noble fir and mid-September for grand fir, until natural seedfall, if stored in a cool, shaded conditions outdoors for one to two months, will yield seeds with as high or higher germinability than seed collected just prior to seed dispersal. Immediate drying and extraction of seed is deleterious to germinability, because the dessication arrests the ripening processes.
3. Stratification of Abies seed frequently, but by no means consistently, increased germination capacities and germination rate.
4. Several seed and cone characteristics taken together seem to offer an easy and effective index of seed maturity. Grand fir and noble fir cones should be ready for harvest when: (a) seed wings are uniformly brown and unattached to the cone scale; (b) cone is light brown in color; (c) embryo length to embryo cavity length is at least 0.90 and the female gametophyte is

firm; and (d) cone specific gravity for grand fir is 0.85 or lower and for noble fir, 0.80 or lower.

5. During seed maturation there is an increase in storage and structural materials within the seed, particularly protein nitrogen and crude fat. Some of the materials are undoubtedly translocated from the senescing cone scales, particularly carbohydrates. Artificial ripening probably facilitates this process, as well as improves seed germination, germination rate and seedling vigor. However, complete translocation of material out of the cone scales is not always possible under artificial ripening treatments.

BIBLIOGRAPHY

Bakuzis, E. and H. Hansen. 1965. Balsam fir, Abies balsamea. A Monographic Review. Univ. of Minnesita Press, Minneapolis. 445 pp.

Barton, L. 1965. Seed dormancy: general survey of dormancy types in seeds, and dormancy imposed by external agents. Encyc. Plant Physiol. 15:699-720.

Bennett, E. 1966. Partial chemical composition of four species of coniferous seeds. For. Sci. 12:316-318.

Ching, T.M. 1960. Seed production from individual cones of grand fir (Abies grandis). J. of For. 58:959-961.

Ching, T.M. and K. Ching. 1962. Physical and physiological changes in maturing Douglas-fir cones and seed. For. Sci. 8:21-31.

Church, T.W. and E. Sucoff. 1960. Virginia pine seed viable two months before natural cone opening. U.S.F.S. N.E. For. Exp. Sta. Res. Note 102, 4 pp.

Cram, W.H. and H. Worden. 1957. Maturity of white spruce cones and seed. For. Sci. 3:263-269.

Crossley, D. 1953. Seed maturity in white spruce. Silv. Res. Notes No. 104.

Danielson, H.R. 1972. Quick tests for determining viability of Douglas-fir seed. Western For. Nursery Coun. and Intermountain For. Nurserymans Assoc. Olympia, Wash. Aug. 8-10.

_____. 1974. Germination, field emergence and storage of noble fir seed (Abies procera). M.S. thesis. Oregon State Univ. 111 pp.

Dekker, R. and G. Richards. 1971. Determination of starch in plant material. J. Sci. Food Agric. 22:441-444.

Dell, T. and F.T. Bonner. 1976. The quantification of germination trends over time using the Weibull function. Annals of Bot. (in press).

Dickmann, D. and T.T. Kozlowski. 1969. Seasonal changes in the macro- and micro-nutrient composition of late stribili and seeds of Pinus resinosa. Can. J. Bot. 47:1547-1554.

Durzan, D. and V. Chalupa. 1968. Free sugars, amino acids and soluble proteins in the embryo and female gametophyte of jack pine as related to climate at the seed source. Can. J. Bot. 46:417-428.

Ebell, L.F. 1968. Variation in total soluble sugars of conifer tissue with method of analysis. Phytochem. 8:227-233.

Edwards, D. 1969. Investigations on the delayed germination of noble fir (Abies procera). Ph.D. thesis. Univ. of Wash. 231 pp.

Eis, S. 1973. Cone production of Douglas-fir and grand fir and its climatic requirement. Can. J. For. Res. 3:61-70.

Eliason, E. and J. Hill. 1954. Specific gravity as a test of ripeness with red pine. Tree Planters Notes 17:1-4.

Fenton, R. and E. Sucoff. 1965. Effects of storage treatments on the ripening and viability of Virginia pine seed. U.S.F.S. N.E. For. Exp. Sta. Res. Note NE-31, 6 pp.

Finnis, J. 1950. Seed maturity in Douglas-fir. B.C. For. Serv. Res. Note 18, 8 pp.

Fowells, H. 1949. An index of ripeness for sugar pine seed. U.S.F.S. Calif. For. and Range Exp. Sta. Res. Note 64, 5 pp.

Franklin, J. 1965. An exploratory study of cone maturity in noble fir. U.S.D.A. For. Serv. Res. Note PNW-21, 12 pp.

_____. 1968. Cone production by upper-slope conifers. U.S.F.S. Pacific N.W. For. and Range Exp. Sta. Res. Paper, PNW-60, 21 pp.

Hansen, R. and D. Boderick. 1968. The fatty acid composition of the lipids from the seeds of Pinus radiata. TAPPI 51:48-51.

Harrar, E. and W. Harlow. 1969. Textbook of Dendrology. McGraw-Hill Book Co. 512 pp.

Holmes, G. and R. Buszewicz. 1958. The storage of seed of temperate forest tree species. Leader article, For. Abst. 19:313-322.

International Seed Testing Association. 1966. International rules for seed testing. Proc. Int. Seed Testing Assoc. 31:69-79.

Katsuta, M. 1961. The synthesis of reserve protein in ripening pine seeds. J. Jap. For. Soc. 43:157-161.

Katsuta, M. and T. Satoo. 1964. Cone development in Pinus thunbergii. J. Jap. For. Soc. 46:166-170.

Konar, R. 1958. A quantitative survey of some nitrogenous substances and fats in the developing embryo and gametophyte of Pinus roxburghii. Phytomorphology 8:174-176.

Krugman, S. 1966. Artificial ripening of sugar pine seeds. U.S.F.S. Pacific S.W. For. Exp. Sta. Res. Paper, PSW-32, 7 pp.

Kulhavy, D. and J. Schenk. 1976. Cone and seed insect damage and prediction of cone production in grand fir in the Potlatch Area of northern Idaho. For., Wildlife and Range Exp. Sta. Univ. of Idaho, Moscow. Contrib. no. 23, 6 pp.

Lavender, D. and W. Engstrom. 1956. Viability of seeds from squirrel-cut Douglas-fir cones. Oregon State Board of For. Res. Note no. 27, 19 pp.

Liu, T.S. 1971. A Monograph of the Genus Abies. Dept. of For., National Taiwan Univ., 608 pp.

Maki, T. 1940. Significance and applicability of seed maturity indices for ponderosa pine. J. of For. 38:55-60.

Mayer, A.M. and A. Poljakoff-Mayber. 1963. The Germination of Seeds. Series of Monogr. on Pure and Applied Biol., vol. 3. Pergammon Press, Internat.

Moore, S. and W. Stein. 1954. A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. J. Biol. Chem. 211:907-913.

Oliver, W. 1974. Seed maturity in white fir and grand fir. U.S.F.S. Pacific S.W. For. and Range Exp. Sta. Res. Paper, PSW-99, 12 pp.

Olson, D. and R. Silen. 1975. Influence of date of cone collection on Douglas-fir seed processing and germination; a case history. U.S.F.S. Pacific N.W. For. and Range Exp. Sta. Res. Paper, PNW-90, 10 pp.

Pfister, R. 1967. Maturity indices for grand fir cones. U.S.D.A. For. Serv. Res. Note, Int.-58, 7 pp.

Rediske, J. 1961. Maturation of Douglas-fir seed--a biochemical study. For. Sci. 7:204-213.

Rediske, J. and D. Nicholson. 1965. Maturation of noble fir seed--a biochemical study. Weyerhaeuser For. Paper 2, 15 pp.

Roe, E.I. 1960. August collected cones yielded poor red pine seed. U.S.F.S. Lake States For. Exp. Sta. Tech. Note 575, 2 pp.

Sarvas, R.O. 1965. Basic studies into the most important factors controlling the quantity and quality of natural seed crops of forest trees. For. Res. Inst. of Finland.

Silen, R. 1958. Artificial ripening of Douglas-fir cones. J. of For. 56:410-413.

Speers, C.F. 1969. Megastigmus specularis Walley infests fir seed from Canada to North Carolins. Tree Planters Notes 20:28-29.

Stoeckeler, J. and G. Jones. 1957. Forest nursery practice in the Lake States. U.S.D.A. Agric. Handbook no. 110, 124 pp.

United States Department of Agriculture, Forest Service. 1974. Seeds of Woody Plants in the United States. U.S.D.A. Agric. Handbook no. 450, 883 pp.

Wakeley, P. 1954. Planting the Southern pines. U.S.D.A. Agric. Monograph no. 18, 233 pp.

Yemme, E. and A. Willis. 1954. The estimation of carbohydrates in plant extracts by anthrone. Biochem. J. 57:508-514.

APPENDIX

Appendix Table 1. Modified Hoagland Solution - amounts of material per liter of solution.

Stock solution A		Stock solution B	
KNO_3	60.6 g	$Ca(NO_3)_2 \cdot 4H_2O$	112.0 g
KH_2PO_4	16.3 g		
$MgSO_4 \cdot 7H_2O$	61.0 g	Stock solution C	
H_3BO_4	0.69 g	KOH	15.0 g
$CuCl_2 \cdot 2H_2O$	0.013 g	EDTA	26.1 g
$MnCl_2 \cdot 4H_2O$	0.43 g	$FeCl_2 \cdot 4H_2O$	17.8 g
$Zn(NO_3) \cdot 6H_2O$	0.055 g		
MoO_3	0.004 g		
NH_4Cl	32.1 g		

Procedure to make stock solution C:

1. Add 300 ml of cold water to container
2. Slowly add KOH pellets while stirring
3. Slowly add EDTA while stirring
4. Add cold water to make one liter
5. Add $FeCl_2 \cdot 4H_2O$
6. Bubble air through solution overnight

Working solution:

A - 200 ml

B - 200 ml

C - 100 ml

Add 0.5 g Dexon to working solution to control fungi

Add 20.0 g sucrose to one liter of working solution

Appendix Table 2. Biochemical constituents in noble fir seed from the Cascade Mountains.

Collection date and storage treatment	Concentration of constituents (mg/g fresh weight)				
	Soluble sugars	Amino acids	Soluble N	Insoluble N	Crude fat
August 6	11.2	2.1	0.56	1.7	18.3
D	-	-	-	-	-
M	-	-	-	-	-
August 20	10.4	3.9	0.94	2.6	23.0
D	-	-	-	-	-
M	8.1	5.6	1.3	5.3	32.7
September 3	12.8	3.4	1.2	14.2	76.4
D	11.7	3.6	1.0	22.1	77.7
M	10.4	3.2	1.2	34.4	60.8
September 18	9.4	2.6	0.72	35.3	80.1
D	6.7	1.9	0.70	34.1	81.6
M	7.9	1.7	0.51	36.9	93.0
September 29	9.2	1.4	0.69	36.4	84.6
D	9.4	1.1	1.1	37.4	92.9
M	11.5	1.3	0.88	38.9	91.8
October 17	13.5	1.0	0.68	43.9	106.2
D	13.9	0.76	0.71	45.6	146.6

Table 2A. Biochemical constituents in noble fir cone scale material from the Cascade Mountains.

Collection date and storage treatment	Concentration of constituents (mg/g fresh weight)[a]			
	Soluble sugars	Amino acids	Soluble N	Insoluble N
August 6	22.5 (124.0)	0.81 (4.5)	0.18 (0.99)	1.1 (6.2)
D	0.64 (0.79)	0.46 (0.57)	0.22 (0.27)	11.5 (14.1)
M	0.77 (3.1)	0.35 (1.4)	0.08 (0.32)	3.5 (13.9)
August 20	23.1 (99.1)	1.10 (4.9)	0.32 (1.4)	1.1 (4.6)
D	0.49 (0.75)	0.49 (0.75)	0.23 (0.35)	7.6 (11.5)
M	0.51 (2.2)	0.27 (1.2)	0.10 (0.42)	3.1 (14.0)
September 3	23.9 (93.4)	1.60 (6.3)	0.47 (1.8)	1.2 (4.8)
D	0.51 (1.1)	0.35 (0.74)	0.26 (0.55)	6.1 (13.0)
M	0.50 (1.5)	0.22 (0.65)	0.12 (0.35)	2.2 (6.4)
September 18	25.7 (79.4)	1.40 (4.4)	0.52 (1.6)	1.3 (4.0)
D	0.53 (1.2)	0.35 (0.78)	0.22 (0.50)	3.1 (6.9)
M	0.52 (1.3)	0.27 (0.69)	0.18 (0.46)	2.2 (5.6)
September 29	10.7 (27.8)	1.10 (2.9)	0.52 (1.4)	2.0 (5.3)
D	0.59 (1.1)	0.35 (0.65)	0.22 (0.42)	4.7 (8.8)
M	0.39 (0.92)	0.41 (0.97)	0.18 (0.43)	3.0 (7.0)
October 17	0.71 (1.0)	0.38 (0.54)	0.09 (0.13)	3.8 (5.4)
D	0.45 (0.59)	0.27 (0.35)	0.12 (0.16)	3.5 (4.6)

[a]Quantities enclosed in parentheses represent mg/g dry weight.

Appendix Table 3. Recovery of glucose from aqueous solution of cone scale extract with Polyclar AT as absorbent.

		Glucose concentration (μg/ml)	Total glucose in solution (μg)
1.	100 μg glucose/ml water solution (10 ml)	92.6	926
2.	100 μg glucose/ml water solution (10 ml) with Polyclar AT (0.5 g)	83.7	837
	90.4% of glucose recovered		
3.	Cone scale extract (10 ml) without Polyclar AT	Unable to read on spectrophotometer	
4.	Cone scale extract (10 ml) with Polyclar AT (0.5 g)	116.2	1162
5.	Cone scale extract (10 ml) plus 5 ml 100 μg/ml glucose solution with Polyclar AT	101.8	1527
	91.8% of glucose recovered based on total glucose expected in water solution (1162 μg + 500 μg = 1662 μg in solution)		

Appendix Table 4. Fresh seed weight of 100 seeds of noble fir and grand fir and specific gravity of cones at the collection times.

	Date of collection	Specific gravity	Fresh seed weight of 100 seeds (g)
Grand fir	July 28	1.03	5.7
	August 10	1.05	5.8
	30	1.03	5.0
	September 3	0.96	4.0
	18	0.82	3.5
	29	Could not determine	
Marys Peak (noble fir)	July 28	0.97	5.2
	August 12	0.99	5.8
	25	0.98	5.2
	September 7	0.89	5.0
	23	0.81	5.8
	October 11	0.64	5.7
Iron Mountain (noble fir)	August 6	0.95	5.4
	20	0.95	4.6
	September 3	0.92	4.6
	18	0.84	4.4
	29	0.64	4.3
	October 17	0.46	4.0

Appendix Table 5. Biochemical constituents in grand fir and noble fir seed (Marys Peak) material.

Collection date and storage treatment	Concentration of constituents (mg/g fresh weight)					
	Soluble sugars	Amino acids	Soluble N	Insoluble N	Crude fat	Starch
			Grand fir			
July 28	17.0	2.2	0.40	0.50	15.0	8.5
D[a]	-	-	-	-	-	-
M[a]	-	-	-	-	-	-
August 10	13.7	2.4	1.0	1.6	27.0	11.7
D	-	-	-	-	-	-
M	-	-	-	-	-	-
August 30	21.1	2.4	1.5	14.6	50.0	12.1
D	-	-	-	-	-	-
M	1.5	0.65	1.2	22.0	94.0	-
September 3	14.7	1.8	0.40	12.5	36.0	12.1
D	14.2	1.1	0.82	28.5	74.0	1.7
M	1.5	0.79	1.3	22.0	70.0	1.7
September 18	14.5	1.5	0.40	13.7	65.0	2.6
D	13.0	1.1	0.47	24.0	121.0	3.9
M	7.2	0.91	0.50	19.8	81.0	4.9
September 29	6.6	1.3	0.49	26.9	110.0	1.3
D	2.2	1.0	0.50	28.9	127.0	1.2
			Noble fir			
July 31	4.6	1.7	0.69	3.8	27.0	3.8
D	-	-	-	-	-	-
M	-	-	-	-	-	-
August 12	9.8	3.3	0.89	7.4	32.0	6.0
D	-	-	-	-	-	-
M	7.5	4.7	1.2	19.9	33.0	1.2
August 25	12.3	4.8	0.99	10.7	29.0	9.8
D	14.9	2.9	1.0	28.0	90.0	4.6
M	14.3	3.2	1.3	28.8	78.0	3.2
September 7	16.7	3.6	0.98	26.2	43.0	7.8
D	16.4	3.0	1.3	31.3	66.0	3.7
M	11.1	1.6	0.51	35.1	85.0	2.0
September 23	12.1	2.1	0.50	38.1	69.0	2.4
D	11.0	1.1	0.70	40.8	110.0	4.4
M	15.6	1.3	0.56	39.8	83.0	2.4
October 11	12.0	1.9	0.37	42.9	223.0	5.7
D	10.5	1.5	0.79	44.4	294.0	6.0

[a] D signifies seed that was stored dry outdoors and M signifies seed that was stored under moist outdoor conditions.

Appendix Table 6. Biochemical constituents in grand fir cone scale and noble fir cone scale (Marys Peak) material.

Collection date and storage treatment	Concentration of Constituents (mg/g fresh weight)[a]				
	Starch	Sugars	Amino acids	Soluble N	Inosluble N
		Grand fir			
July 28	1.4 (5.2)	17.0 (64.0)	2.1 (8.1)	0.53 (2.0)	3.5 (13.1)
D	0.0 (0.0)	6.0 (7.0)	5.8 (6.8)	0.59 (0.69)	10.1 (11.9)
M	0.0 (0.0)	1.1 (1.5)	0.81 (1.1)	0.25 (0.25)	12.9 (17.6)
August 10	0.45 (1.5)	13.7 (45.0)	2.4 (7.8)	0.59 (1.9)	3.7 (12.3)
D	0.0 (0.0)	4.8 (5.6)	1.9 (2.2)	0.36 (0.42)	7.9 (9.3)
M	0.10 (0.14)	1.2 (1.7)	0.58 (0.82)	0.24 (0.24)	10.9 (15.3)
August 30	0.53 (1.5)	21.1 (60.0)	2.4 (7.0)	0.50 (1.4)	3.2 (9.2)
D	0.44 (0.52)	4.4 (5.2)	1.7 (2.9)	0.50 (0.59)	8.2 (9.7)
M	0.20 (0.34)	1.5 (2.5)	0.65 (1.1)	0.29 (0.29)	10.9 (18.5)
September 3	0.88 (2.4)	15.7 (45.0)	2.0 (5.9)	0.26 (0.75)	1.0 (2.9)
D	0.85 (0.97)	4.7 (5.6)	1.2 (1.3)	0.50 (0.55)	5.2 (5.7)
M	0.10 (0.13)	0.65 (0.90)	0.22 (0.30)	0.20 (0.27)	3.4 (4.6)
September 18	0.06 (0.14)	8.4 (20.0)	1.3 (3.0)	0.30 (0.71)	1.5 (4.8)
D	0.0 (0.0)	2.4 (2.9)	0.50 (0.60)	0.29 (0.35)	3.4 (4.2)
M	0.0 (0.0)	0.61 (2.5)	0.44 (1.0)	0.23 (0.32)	2.0 (3.5)
September 29	0.0 (0.0)	6.1 (7.0)	0.90 (1.0)	0.25 (0.29)	3.6 (4.2)
D	0.0 (0.0)	1.9 (2.1)	0.50 (0.60)	0.18 (0.31)	2.9 (4.1)
		Noble fir			
July 31	0.24 (1.2)	33.5 (167.0)	3.4 (17.0)	0.39 (1.9)	1.1 (5.6)
D	0.0 (0.0)	0.92 (1.2)	0.67 (0.84)	0.24 (0.31)	11.1 (13.8)
M	0.0 (0.0)	0.57 (0.99)	0.28 (0.48)	0.14 (0.24)	5.3 (9.0)
August 12	0.10 (0.56)	34.6 (195.0)	2.0 (11.0)	0.26 (1.5)	0.9 (5.2)
D	0.0 (0.0)	0.64 (0.86)	0.42 (0.58)	0.14 (0.19)	6.0 (8.9)
M	0.0 (0.0)	0.59 (1.4)	0.98 (2.3)	0.30 (0.78)	3.9 (9.5)
August 25	0.21 (0.88)	41.4 (174.0)	3.3 (14.0)	0.28 (1.2)	0.8 (3.2)
D	0.16 (0.30)	0.40 (0.75)	0.27 (0.47)	0.10 (0.19)	4.3 (8.2)
M	0.0 (0.0)	0.47 (1.0)	0.22 (0.51)	0.13 (0.28)	1.5 (3.2)
September 7	0.60 (2.2)	38.5 (143.0)	1.9 (6.0)	0.30 (1.1)	0.87 (3.2)
D	0.08 (0.12)	0.57 (0.87)	0.37 (0.56)	0.10 (0.15)	3.6 (5.6)
M	0.42 (0.86)	0.71 (1.5)	0.22 (0.45)	0.09 (0.19)	2.0 (4.1)
September 23	0.13 (0.39)	43.5 (131.0)	2.1 (6.0)	0.35 (1.0)	1.4 (4.3)
D	0.0 (0.0)	0.70 (1.5)	0.19 (0.42)	0.08 (0.19)	2.6 (5.6)
M	0.0 (0.0)	0.54 (0.92)	0.21 (0.58)	0.14 (0.39)	1.7 (4.3)
October 11	0.01 (0.02)	10.0 (21.0)	1.4 (3.0)	0.16 (0.33)	2.3 (4.8)
D	0.0 (0.0)	0.57 (0.84)	0.19 (0.28)	0.07 (0.10)	2.2 (3.3)

[a] Quantities enclosed in parentheses represent mg/g dry weight.